Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 40. MARCH, 1939. No. 3.

SECTION MEETINGS

CLEVELAND	
Western Reserve Univ. Inst. of Pathology	January 13, 1939
ILLINOIS	
University of Chicago	March 7, 1939
MINNESOTA	
University of Minnesota	February 15, 1939
MISSOURI	
St. Louis University	February 8, 1939
Washington University	March 8, 1939
PACIFIC COAST	
Stanford-Lane Hospital	February 1, 1939
PEIPING	
Peiping Union Medical College	January 12, 1939
WESTERN NEW YORK	
University of Rochester	February 18, 1939

10397

Rôle of Adrenals in Blood Pressure Reaction to Anoxia During Insulin Hypoglycemia.

R. C. INGRAHAM AND E. GELLHORN.*

From the Department of Physiology and Psychiatry of the University of Illinois, College of Medicine.

Gellhorn, Ingraham and Moldavsky[1] reported recently that on inhalation of 6% oxygen for brief periods (1-3 minutes) the blood

* Aided by The John and Mary R. Markle Foundation.

1 Gellhorn, E., Ingraham, R., and Moldavsky, L., *J. Neurophysiol.*, 1938, **1**, 301.

pressure rises greatly during insulin hypoglycemia whereas at normal blood sugar level the blood pressure is either unchanged or rises only a few millimeters. The reaction is reversible when the original blood sugar level is restored by the injection of glucose. Kraines and Gellhorn[2] showed that this reaction is observed under similar conditions in the human. This phenomenon was interpreted as an indication of a synergistic action between the effects of anoxia and those of hypoglycemia on the central nervous system. Since the latter seems to depend almost exclusively on the consumption of carbohydrates as indicated by the fact that the R.Q. of the brain approaches unity (Lennox,[3] Dickens and Simer[4]) a reduction in the glucose level of the blood is apt to curtail the oxidative metabolism of the central nervous system and to make it more susceptible to the effects of a diminished oxygen supply induced by the inhalation of gas mixtures low in oxygen. It is assumed that this diminution in the oxygenation of nervous tissues is the cause of the excitation of the sympathico-adrenal system which commonly accompanies states of anoxia. The question, therefore, presents itself to what extent the greatly increased blood pressure reaction to low oxygen in the hypoglycemic state is due to purely nervous impulses over the sympathetic and to humoral effects due to an increase in the liberation of adrenin. The following experiments were devised to decide this question.

Methods. Experiments were carried out on 7 dogs under chloralosane or amytal anesthesia. Insulin was injected intravenously (3 to 12 units per kilo); 6.2% oxygen was inhaled from Douglas bags for periods of 3 minutes before the injection of insulin and at various intervals during the hypoglycemia. As soon as a greatly increased blood pressure reaction occurred the adrenal veins were ligated bilaterally and the test was repeated.

Results. The results were uniform in all experiments and seem to indicate that the adrenal glands do not play any significant part in the increased blood pressure reaction to low oxygen observed in the hypoglycemic state. In the first curve of Fig. 1 the blood pressure response to 6.2% oxygen is practically the same before and after the ligation of the adrenals, the blood sugar values being 66 and 63 mg % respectively. The second curve of Fig. 1 shows an experiment in which during the time which elapsed between the last test carried out before and the first test carried out after elimination of the adrenals the blood sugar fell from 75 mg % to 68 mg %. In spite of

[2] Kraines, S., and Gellhorn, E., in press.

[3] Lennox, W. G., *Arch. Neurol.-Psychiat.*, 1931, **20**, 719.

[4] Dickens, F., and Simer, F., *Biochem. J.*, 1931, **25**, 985.

the elimination of the adrenal glands the blood pressure reaction to the inhalation of 6.2% oxygen increased greatly. Similar was the result in other experiments. The blood pressure reaction was dependent on the blood sugar level but not on the presence of the adrenal glands. When the former fell the reaction increased; when the blood sugar rose the blood pressure response was diminished.

An interesting experiment is reproduced in the third curve of Fig. 1. The blood sugar level before and after ligation of the adrenals was practically identical (39 and 38 mg % respectively) but much lower than in the experiment shown in the top curve of Fig. 1. It is seen from the graph that the reaction was greatly increased after elimination of the adrenals. This result is in harmony

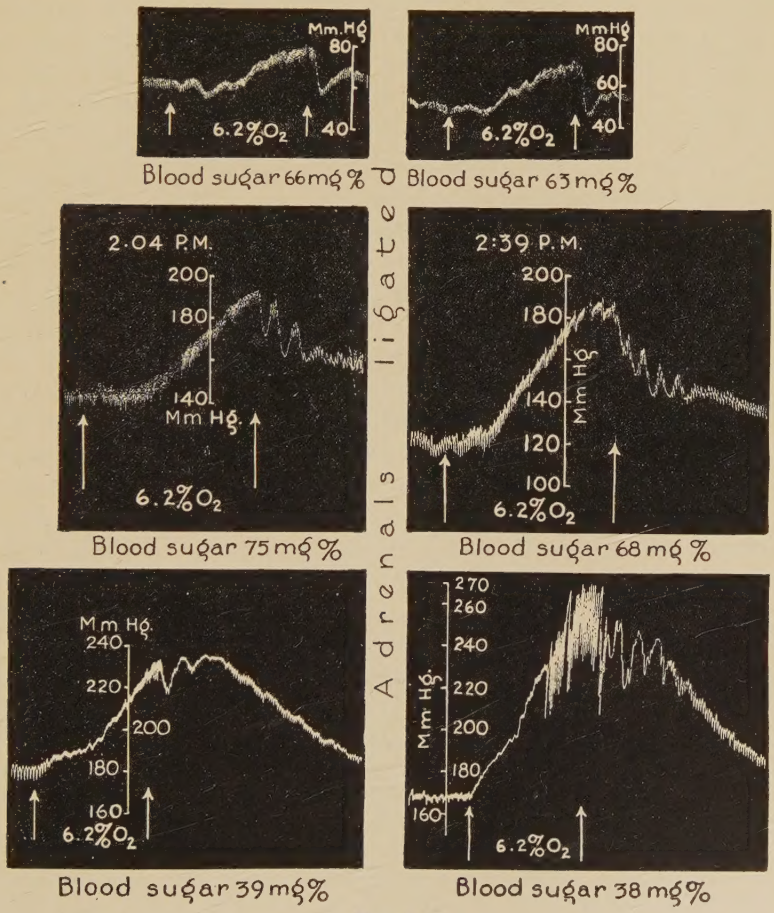


FIG. 1.

Experiments on narcotized dogs showing the blood pressure response to the inhalation of 6.2 % oxygen before and after ligation of both adrenals.

with the earlier experiments of Gellhorn, Ingraham and Moldavsky,[1] who observed that at low blood sugar levels the blood pressure response to 6.2% oxygen increased with increasing duration of the hypoglycemic state. It may, therefore, be said that the two conditions, *i. e.*, the blood sugar level and the duration of the hypoglycemic state at a given blood sugar concentration which were found to determine the blood pressure reaction to low oxygen in the normal animal also determine this reaction after elimination of the adrenals. The latter do not seem to play any part in the blood pressure reaction.

It still remains to be decided if an anoxia of the extent and duration employed in these experiments is a sufficient stimulus to cause the liberation of significant quantities of adrenalin. An approach to this problem has been made by causing unnarcotized rabbits to inhale 6.2% oxygen for 3 minutes and following the blood sugar level over a period of 30 minutes. It was found that at the end of the 3-minute period of anoxia the blood sugar level was raised by an average of 12.8% and showed a maximum (average rise of 24%) 13 minutes later. Therefore, under these conditions, this period of anoxia is a sufficient stimulus for adrenalin liberation but because of the species difference and lack of narcosis, these results cannot be applied directly in interpreting the experiments described here. But if we take into account the fact that according to unpublished observations of one of us (Gellhorn) the effectiveness of anoxia to increase adrenin secretion as measured by the blood sugar response is greatly augmented during insulin hypoglycemia it seems to be probable that adrenin is secreted to an increased extent during the anoxia period in our experiments on dogs. But the amount liberated is insufficient to account for the observed blood pressure reaction.

Studies on the blood pressure reaction produced by the intravenous injections of adrenalin compared with the response to 6.2% oxygen during insulin hypoglycemia throws further light on this problem. It was found that the injection of 0.05 mg adrenalin produces a blood pressure rise similar to that seen in the last graph of Fig. 1. The reaction, however, was of much shorter duration than that observed during inhalation of 6.2% oxygen at a low blood sugar level. It is, therefore, necessary to assume that a multiple of this amount of adrenin must be secreted in order to account for the blood pressure changes. This may not appear impossible in the light of the investigations of Cannon and Rapport,[5] who found the adrenin output greatly increased under stress. But the experiments reported in this paper show clearly that the amount of adrenin which may be se-

[5] Cannon, W. B., and Rapport, D., *Am. J. Physiol.*, 1921, **58**, 308.

creted during such a brief anoxia period is insufficient to account for the greatly increased blood pressure response to anoxia. This reaction is, therefore, under the conditions of our experiments exclusively or almost completely due to increased sympathetic discharges and not to humoral factors.

It is interesting to note that our results as to the relation of sympathetic discharges to the secretion of adrenin are similar to those of Heymans[6] and Nowak[7] who studied the significance of adrenin secretion for the blood pressure response in carotid sinus reflexes. They found that the rise of blood pressure following a decrease of the pressure in the carotid sinus was quantitatively unaltered by the elimination of the adrenals although it is known that under these conditions the adrenin secretion increases (Heymans,[8] and others).

Summary. The blood pressure rise which increases in response to the inhalation of 6% oxygen when the blood sugar falls during the course of insulin hypoglycemia is not influenced by bilateral ligation of the adrenals. It is, therefore, concluded that under the conditions of our experiments an increased secretion of adrenin does not account for the greatly increased blood pressure response. The latter seems to be due to increased sympathetic discharges only.

10398

Concentration and Possible Significance of Histaminase in the Human Placenta.

D. N. DANFORTH. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Occasional reports have appeared which indicate the presence of histamine and its precursors in the placenta.[1-4] This seems logical because of the amount of degenerating tissue present in the placenta during the later months of pregnancy. Since histamine may be

6 Heymans, C., *Compt. Rend. Soc. Biol.*, 1933, **114**, 148.

7 Nowak, St., *Compt. Rend.*, 1934, **115**, 1731.

8 Heymans, C., *Arch. internat. Pharm. Therap.*, 1929, **35**, 269.

1 Harding, V. J., and Fort, C. A., *J. Biol. Chem.*, 1918, **35**, 29.

2 Bartholomew, R. A., and Kracke, R. R., *Am. J. Obst. and Gynec.*, 1932, **24**, 797.

3 Bartholomew, R. A., and Parker, F., *Am. J. Obst. and Gynec.*, 1934, **27**, 67.

4 Hofbauer, J., *Am. J. Obst. and Gynec.*, 1926, **12**, 159.

formed in amounts theoretically capable of stimulating a sensitive uterus, it seemed consistent to assume that the placenta might contain appreciable amounts of the enzyme histaminase for the purpose of inactivating histamine and thereby rendering it inert as an oxytocic. When histaminase was found[5] to be present in the human placenta in extremely variable amounts the question arose whether the enzyme content of the organ might be related to the efficiency of uterine contractions. The study was then extended, and at the conclusion of the analyses for histaminase the clinical labor histories were examined and correlated.

One hundred eighty-two placentae were used in this study. Each placenta was weighed immediately after delivery, multiple incisions were made, and the organ was then placed in acetone. From this point the preparation and assay of histaminase were conducted according to the method of Best and McHenry.[6] The placentae were thoroughly ground and mixed before aliquots were taken for the preparation of the powders. All assays were made in duplicate. The original incubation of each placenta was conducted using 1000 mg of powder to 1 mg of ergamine acid phosphate. If inactivation occurred in both flasks, a second incubation was started using 750 mg of placenta powder to 1 mg of ergamine. If this amount was sufficient to completely inactivate the contained histamine, the amount of powder was then reduced to 500 mg, 250 mg, and so on, with reductions of 50 mg at each subsequent assay, until no inactivation occurred. The number of units of histaminase present in 1000 mg of placenta powder was then computed, and the approximate amount in the entire placenta determined. In this way it is possible to determine the approximate number of units of histaminase in a given placenta and the values obtained, though not entirely precise, are sufficiently accurate for purposes of comparison.

In Table I a *normal labor* was considered as one in which uterine contractions were of average or better than average efficiency, and in which the duration fell within the limits of error for the particular group of patients presented. In those patients in whom labor was *"induced,"* one or more of the following methods was used: castor oil, enemata, quinine, and stripping or rupture of the membranes. In women who were given a *test of labor,* strong uterine contractions were allowed to progress until the obstetrician was convinced that there was disproportion sufficient to preclude delivery from below.

[5] Danforth, D. N., and Gorham, F., *Am. J. Physiol.*, 1937, **119**, 294.

[6] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

TABLE I.

Labor history	No. of specimens	Avg No. of units of histaminase per entire placenta
Normal	71	Less than 31 ± 1.0
Shorter than normal, not induced	32	" " 31 ± 2.4
Induced, good response	31	" " 32 ± 3.6
Longer than normal, the contractions good	24	" " 25 ± 1.1
Caesarian section following test of labor	8	" " 26 ± 0.7
Caesarian section before onset of labor	5	More than 43 ± 2.7
Longer than normal, sluggish contractions	9	" " 45 ± 1.5
*Uterine inertia	2	110 and 198
Total specimens	182	

*These 2 specimens were obtained from women who were past term and in whom labor had started spontaneously and had continued with very poor contractions for 40 and 44 hours respectively. Dilatation of the cervix did not occur. Labor was terminated at the end of this time by Caesarian section.

When computed statistically many of the differences cited are shown to be barely significant. Because of the rather obvious grouping, however, there appears to be some correlation between the efficiency of uterine contractions during parturition and the amount of histaminase in the placenta. In those women in whom contractions were poor, excessive amounts of histaminase were found; where contractions were strong and allowed to proceed for longer than the normal time the placentae were relatively poorer in histaminase.

These findings may be interpreted in several ways. *First,* one may assume that the efficiency of the laboring uterus is inversely proportional to and dependent upon the histamine-destroying power of the uterus. As this histamine-destroying power is lost, labor may proceed unimpeded. This hypothesis would indicate that the presence of histamine is an essential for the maintenance of normal parturition, and might even point to this factor as the etiologic agent in the spontaneous onset of labor. Since histamine is known to be oxytocic and since its liberation by degenerating tissue is quite probable, such a hypothesis is not irrational. However, there are several reasons why this theory is probably untenable. Marcou[7] has found that the blood histamine level declines steadily in the few days before labor, and does not return to normal until after delivery. If the placentae were a source of histamine in amounts sufficient to precipitate labor, one might expect a rise in the blood level before parturition. Further, we[8] have been unable to initiate premature labor in guinea pigs and rabbits by the administration of histamine in physiologic doses. An-

7 Marcou, L., and Atanasiu-Vergu, *Bull. l'Acad. Med. de Roumaie,* 1937, **3**, 1.
8 Ivy, A. C., and Danforth, D. N., unpublished data.

other objection is that this theory does not consider those autacoids, especially estrin and progestin, which have been shown to play a major rôle in changes in uterine motility.

Second, the inverse relationship of placental histaminase to efficiency of labor pains may be due to the unknown factor or factors which initiate and promote the continuance of labor. That is, the observed changes may be an effect and not a cause.

Third, and most likely, the histaminase content of the placenta is in no way *directly* related to uterine contractions, but may be thought of rather as an index of the amount of active, functioning parenchymatous tissue in the placenta. If this interpretation is accepted, then the rôle of the placenta in the onset and maintenance of parturition becomes more apparent, and physiologic senescence is indicated as a salient, if not causal, factor in the onset and maintenance of labor.

Summary. Although the amount of histaminase in the placenta shows some correlation with the efficiency of uterine contractions, the evidence is not sufficiently definite to warrant such a conclusion.

10399

The Relation of Bile and Pancreatic Juice to Duodenal Ulcer in Dogs.

W. H. BACHRACH, C. R. SCHMIDT AND J. M. BEAZELL. (Introduced by A. C. Ivy.)

From the Department of Physiology, Northwestern University.

Among the numerous investigators who have worked with complete external biliary fistulas in dogs, the majority[1–6] have reported a high incidence of duodenal ulcer. Puestow[7] makes no mention of ulceration in a series of dogs with a segment of the duodenum containing the orifice of the common duct exteriorized, but he is quoted by Blanck[5] as having stated that several of his animals died rather

1 Berg, Johnston and Jobling PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 334.
2 Berg and Jobling, *Arch. Surg.*, 1930, **20**, 997.
3 Kim and Ivy, *J. A. M. A.*, 1931, **97**, 1511.
4 Hawkins and Whipple, *J. Exp. Med.*, 1935, **62**, 599.
5 Blanck, *Surg. Gyn. and Obst.*, 1935, **61**, 480.
6 Hanke, *Arch. f. klin. chir.*, 1937, **187**, 675.
7 Puestow, *Arch. Surg.*, 1931, **23**, 1013.

suddenly with perforated peptic ulcers. Rous and McMaster[8] and Kocour and Ivy[9] are silent on the occurrence of ulcer in their dogs, and one assumes that they did not find any. Brunschwig, Bissell and Andrews[10] state definitely that in their chronic biliary fistula dogs they found no changes in the gastric or intestinal mucosa.

It is interesting that no ulcers were noted by those two groups of investigators[8, 9] who developed a good method for the return of bile. This phase of the problem was approached by Blanck[5] who reported experiments in biliary fistula dogs which he interpreted as proving that the loss of bile results in peptic ulceration, and that the formation of these ulcers is prevented by adding dog bile to the diet of such animals. However, we believe that one cannot accept the author's conclusions on the basis of his data, because obviously, the series of dogs is small, the observation time is very short, and the difference between the control and bile-fed groups is too slight to be significant.

In the present communication, we wish to present a summary of our observations on the incidence of duodenal ulcer in (a) 15 dogs with complete external biliary fistulas prepared according to the method of Rous and McMaster as modified by Kocour and Ivy, and (b) 19 dogs with exclusion of pancreatic juice by separation of the pancreas from the duodenum.

A. *Incidence of ulcer in biliary fistula dogs.* Our 15 dogs to which bile was returned survived from 16 to 175 days after preparation of the bile fistula, only one dog surviving less than 4 weeks. The longest survival recorded by Blanck was 24 days. In our series, chronic ulcers of the duodenum occurred in only 2 dogs; in both of these the ulcer was directly responsible for death by perforation into the peritoneal cavity. The ulcers came as a surprise finding at autopsy because of the excellent general condition of the animals up to a day or two preceding death.

It is interesting that in the 2 dogs which died with chronic ulcers, the bile was returned via a long rubber tube passing through the common duct and terminating in the small intestine about 50 cm distal to the papilla of Vater. This raises the question whether the absence of bile from the upper intestine, rather than bile loss *per se*, is the important factor in protecting the duodenal mucosa from ulceration. The literature bearing on this point is too extensive to be discussed here. We feel justified at the present time in concluding

8 Rous and McMaster, *J. Exp. Med.*, 1923, **37**, 11.

9 Kocour and Ivy, *Am. J. Physiol.*, 1938, **122**, 325.

10 Brunschwig, Bissell, and Andrews, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 41.

that the return of bile to the upper gastrointestinal tract, either by mouth or by an indwelling tube, prevents the formation of duodenal ulcers.

B. *Incidence of ulcer following pancreatic exclusion.* With regard to the question of pancreatic exclusion, we have observed 19 dogs in which the pancreas was separated from the duodenum; in this preparation there is, of course, no entrance of pancreatic juice into the intestine.

The dogs survived from 21 to 720 days after the operation, with malnutrition varying from slight to severe (50% of body weight); in only one instance was a duodenal ulcer observed at autopsy. These findings agree with those of Ivy,[11] who reported one case of true ulceration in 24 dogs with ligated pancreatic ducts. The problem of ulcer formation following external drainage of pancreatic juice involves not only exclusion of the juice from the duodenum, but also the very serious matter of fluid and salt loss, and our present report is not concerned with this aspect of the problem. It may be accepted as an established fact that duodenal ulcer only rarely results from absence *per se* of pancreatic juice from the upper intestine.

Summary. Of 15 dogs with complete external drainage of bile, all being supplied with bile by mouth or by a tube entering the upper intestine, duodenal ulcer was observed in 2 cases; only one instance of ulcer was noted in 19 dogs with exclusion of the pancreatic juice by separation of the pancreas from the duodenum.

10400

Development of Refractoriness to Enterogastrone Preparations.*

J. S. GRAY AND E. WIECZOROWSKI. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

It has been established that after prolonged administration animals may become refractory to crude hormone preparations, particularly to those of hypophyseal origin, which are water-soluble and protein-like in nature. It has not been demonstrated that animals may become

[11] Ivy, *Arch. Int. Med.*, 1920, **25**, 6.

* Aided in part by a grant from the Committee on Research in Endocrinology of the National Research Council.

refractory to preparations of the water-soluble duodenal hormones, although allergic types of reactions have been observed.[1]

Preparations of enterogastrone, a duodenal chalone which inhibits gastric motility and secretion,[2, 3] have been found to induce a refractory state in certain dogs. Of 8 dogs with pouches (vagotomized) of the entire stomach that have been repeatedly injected over long periods of time with enterogastrone preparations, 2 have very definitely become refractory within one month, whereas the remaining 6 dogs have given no indication of altered tolerance over periods varying from several months to several years. In the case of one of these animals it has been possible to demonstrate that the tolerance is directed toward the extracts, presumably impurities, and not toward the chalone elaborated by the animal itself.

During the months of April and May, 1936, 2 total pouch dogs, T-1 and T-2, were each injected 5 times with enterogastrone preparations of known potency. The resulting degree of inhibition of continuous histamine secretion was consistent and comparable in the 2 animals. Early in June attempts to assay chemically separated fractions of these preparations were unsatisfactory because of inconsistency in the responses of the 2 animals. Accordingly, a standard preparation to which both animals had previously responded was again administered to both dogs. Dog T-2 failed to respond, whereas dog T-1 exhibited its original degree of inhibition. This procedure has been frequently repeated and dog T-2 has never again exhibited an inhibitory response to the administration of enterogastrone preparations. Fortunately the degree of inhibition produced by the intraduodenal administration of olive oil had been determined in this animal before it had become refractory. A second determination performed after the dog had become refractory yielded practically the same degree of inhibition as had previously been obtained. These results demonstrated clearly that although the animal had become refractory to the enterogastrone preparations, it had not become refractory to the chalone which was elaborated by its own duodenal mucosa when exposed to the action of fat.

In September, 1937, dog T-4 received several injections of enterogastrone to which it gives responses comparable to those of the other animals in use at the time. Later when attempts were made to assay preparations which had been subjected to purification procedures,

1 Voegtlin, W. L., Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1934, **110**, 198.

2 Gray, J. S., Bradley, W. B., and Ivy, A. C., *Am. J. Physiol.*, 1937, **118**, 463.

3 Ivy, A. C., and Gray, J. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**, 405.

TABLE I.
Development of Tolerance to Enterogastrone Preparations.

Dog	Date	Substance Assayed	Quantity	% Inhibition
T-2	4/15/36	Prep. T-5	1 unit	—60
T-2	5/7	Olive oil	50 cc	—20
T-2	6/16	Prep. T-5	1 unit	— 7
T-2	6/17	Olive oil	50 cc	—23
T-4	9/11/37	Prep. T-43	1 unit	—54
T-4	11/22/38	" W-5	100 mg	+ 4
T-4	11/23	" W-5	100 "	+11
T-4	12/7	" W-5	100 "	—16

it was found that this animal yielded results quite inconsistent with other animals. This animal was then used for a considerable period for other studies. In November, 1938, a modified method of assaying enterogastrone against histamine secretion was studied. It was found that dog T-4 failed to respond to 100 mg doses of a standard preparation to which 3 other dogs gave an average (7 assays) of 74% inhibition. The pertinent data demonstrating that dogs T-2 and T-4 had become refractory to enterogastrone preparations are exhibited in Table I.

Although it has been more difficult to demonstrate conclusively, we have received the impression that unoperated dogs occasionally develop a tolerance after they have been repeatedly injected with small doses of enterogastrone for purposes of assaying their potency in inhibiting gastric motility.

Since the methods for assaying enterogastrone have always required chronic animals, the development of tolerance has been a hindrance to reliable assays. The purpose of this report is to call attention to this difficulty for the benefit of those who may be engaged in assaying duodenal extracts.

Conclusions. (1) After repeated injections dogs occasionally become refractory to preparations of the duodenal chalone, enterogastrone. (2) This refractoriness is probably due to impurities in the extracts, since continued response to the chalone elaborated by the animal's own duodenum has been observed.

10401

Androgenic Function of the Adrenals in the Immature Male Castrate Rat.*

M. W. Burrill and R. R. Greene. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

In the adult male rat, castration invariably results in atrophy of the prostate within 4 or 5 days. In the immature male rat, however, castration is not accompanied by atrophy of the prostate. Price[1] found that the rat prostate will develop in an essentially normal manner in the absence of the gonads and that the functional integrity of the ventral prostate is maintained for a certain period after early removal of the gonads. Howard[2] confirmed Price's observations on the rat and suggested that a transitory andromimetic function of the adrenal may be responsible for the maintenance of the immature prostate in the absence of the testes. Davidson and Moon[3] found that administration of adrenocorticotropic substance to the immature castrated male rat caused hypertrophy of the adrenal cortex and also produced a gross and histological stimulation of the prostate.

The present experiment was undertaken to determine directly whether or not the immature male adrenal possesses an andromimetic function.

Immature male albino rats from our stock colony were used in the experiment. Litters were divided into 3 groups: (1) castrate at 16 days, (2) castrate at 16 days and adrenalectomized at 21 days, and (3) adrenalectomized at 21 days. All the animals were killed at 26 days of age. The ventral prostates were removed, fixed in Bouin's fluid, sectioned and stained with hematoxylin and eosin. An additional group of animals (not litter mates) were castrated at various ages (4 to 18 days) and killed at 26 days of age. Their ventral prostates were also removed and treated for microscopic examination.

All operations were done under ether anesthesia, particular care being taken to keep the anesthesia light for adrenalectomy. Powdered milk, bread, Purina dog checkers and 2% NaCl in tap water

* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

1 Price, D., *Am. J. Anat.*, 1936, **60**, 79.

2 Howard, E., *Am. J. Physiol.*, 1937, **119**, 339.

3 Davidson, C. S., and Moon, H. D., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 281.

were supplied to all the animals. Part of the litters were allowed to remain with the mother for the duration of the experiment inasmuch as the double shock of adrenalectomy and weaning appeared to increase mortality. The animals were generally in good condition when they were killed.

The normal ventral prostate of the mature rat consists of large acini with a delicate interacinous stroma. The acinar epithelium is composed of high, columnar cells with basal nuclei and characteristic light areas just distal to the nuclei. The presence of the light area which appears with ordinary fixation and staining technic and which represents the site of the Golgi body, has been accepted as the criterion for secretory activity of the prostate.[4] The ventral prostate, following castration, presents a highly atrophic appearance: the acini are reduced in size, the stroma is apparently increased in amount, the epithelium is changed to a low, cuboidal type, sometimes scarcely wider than the nuclei, and the light areas are absent.

In the normal 26-day-old male, the ventral prostate presents an appearance which is practically identical with that of the normal adult except for the generally smaller size of the acini. After castration at various ages up to 21 days (Price), the prostates are temporarily maintained in a state which approaches the normal. The duration of this period of maintenance is dependent on the age at which castration is performed. According to Price, the prostate is maintained in a functional state for 10 to 20 days following castration between the ages of 16 to 21 days. The term "functional" applies mainly to the presence of light areas inasmuch as some atrophy of the gland may occur: the epithelium may be somewhat lower than normal, although not as low as in the adult castrate; the acini may be somewhat smaller than normal and the stroma may be slightly increased in amount.

In 18 adrenalectomized castrates, the ventral prostates consistently showed marked atrophy identical with that found in the adult castrate. In 6 adrenalectomized non-castrates the gland was histologically normal. In the 13 animals which were castrated but not adrenalectomized, the prostates were essentially normal in appearance. The height of the epithelium, size of acini and amount of stroma varied slightly in individual cases. The distribution of the light areas also varied somewhat. In the prostates of 4 of these castrate animals light areas were not found. However, none of the prostates of the plain castrates presented the true castration picture which was found in the prostates of the adrenalectomized castrates.

[4] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 71.

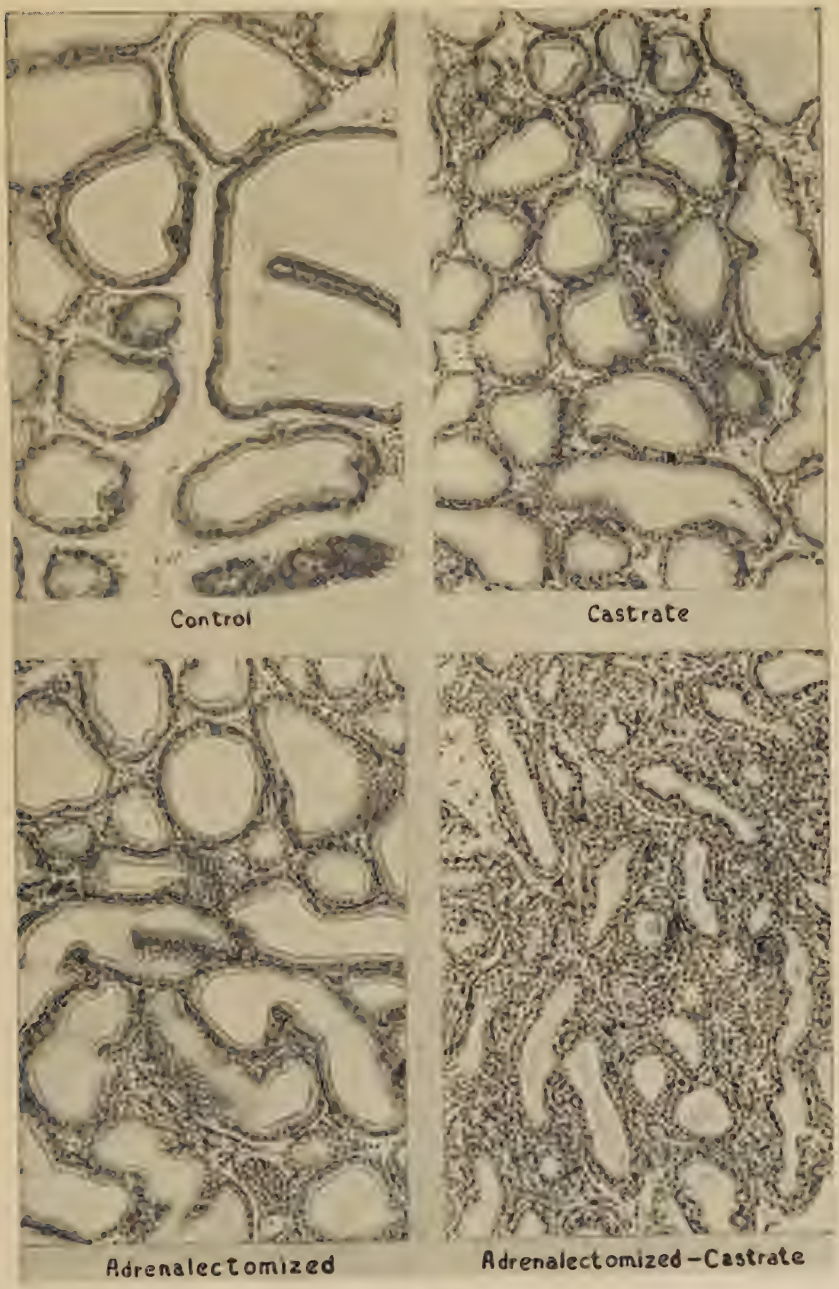


FIG. 1.
Sections of the ventral prostate.

In the 11 animals which were castrated at different ages (4 to 18 days), the prostates at 26 days of age were essentially normal in appearance. Some variation in the distribution of the light areas also occurred in these animals.

The experimental results clearly indicate that the adrenals of the immature male rat, in the absence of the gonads, are responsible for the maintenance of the ventral prostate gland in a normal or nearly normal condition. When the testes alone are removed, the prostate remains intact; when testes and adrenals are both removed, the prostate undergoes involution. That this latter effect is specific and not the result of general debility due to the adrenalectomy has been demonstrated inasmuch as removal of the adrenals alone produces no change in the prostates.

The fact that removal of the immature adrenals alone does not alter the condition of the prostate indicates that normally the adrenals are not appreciably involved in the development and maintenance of the prostate. The testes apparently produce sufficient androgen to maintain them. However, in the absence of the gonads, the immature adrenals are capable of maintaining the prostate in a "functional" state. This capacity of the adrenal apparently exists only in the immature animal since, after a certain age, prostatic atrophy always follows castration. How can this change be explained? Either the adrenal, in the course of its development, loses its andromimetic function or this function is retained, in which case the amount of androgen produced by the mature adrenal is probably insufficient to maintain the mature prostate. The latter alternative seems the more probable since certain substances having androgenic power have been isolated from the adult adrenals. Adrenosterone was isolated from the adrenal by Reichstein,[5] who also showed that it has androgenic powers. Progesterone has been isolated from the adrenal by Beall and Reichstein.[6] Its androgenicity has been established by Lamar[7] and by Greene, Burrill and Ivy.[8] The fact that other adrenal substances, such as corticosterone and desoxycorticosterone, are chemically similar to progesterone suggests that they also may be androgenic, at least to a slight degree.

---

[5] Reichstein, T., *Helv. Chem. Acta*, 1936, **19**, 223.

[6] Beall, O., and Reichstein, T., *Nature*, 1936, **142**, 479.

[7] Lamar, J. K., *Anat. Rec.*, 1937, **70**, Suppl. p. 45.

[8] Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrinology*, 1939, **24**, 351.

10402

Does Renin Play a Rôle in the Maintenance of Normal Blood Pressure?*

GEORGE E. WAKERLIN AND GEORGE R. CHOBOT.

From the Department of Physiology, College of Medicine, University of Illinois.

Research during the past few years points strongly to the probability that the Goldblatt[1] type of experimental renal hypertension results from the liberation by the constricted kidney of some effective substance which directly or indirectly acts as a pressor agent.[2-7] As a consequence, interest in the pressor substance, renin, which was first extracted from the kidney by Tigerstedt and Bergmann[8] has been revived.

Although there is scant, circumstantial evidence that renin is involved in the pathogenesis of experimental renal hypertension,[9, 10] no evidence is available at present for the possibility that renin plays a rôle in the maintenance of normal arterial blood pressure. We have investigated the latter possibility by determining (1) the renin content of the kidneys and the blood pressures of normal dogs, (2) the effect of splanchnic stimulation, and (3) the effect of induced hypotension on the renin content of the kidneys of normal dogs. Splanchnic stimulation and hypotension constitute conditions of physiological stress which conceivably might affect the formation and secretion of renin.

Two methods of preparing renin were used. One consisted of extracting the ground kidney with 0.2% HCl in physiological salt solution, centrifuging, storing overnight in the ice box, and recentri-

* This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

1 Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

2 Page, I. H., *Am. J. Physiol.*, 1935, **112**, 166.

3 Elaut, L., *Comp. rend. Soc. biol.*, 1936, **122**, 126.

4 Goldblatt, H., Gross, J., and Hanzal, R. F., *J. Exp. Med.*, 1937, **65**, 233.

5 Freeman, N. E., and Page, I. H., *Am. Heart J.*, 1937, **14**, 405.

6 Child, C. G., and Glenn, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 217.

7 Alpert, L. K., Alving, A. S., and Grimson, K. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 1.

8 Tigerstedt, R., and Bergmann, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

9 Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

10 Prinzmetal, M., and Friedman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

fuging. The other method was that of Grossman[11] which involved extracting alcohol-ether desiccated kidney pulp with 0.5% $NaHCO_3$ in physiological salt solution.

The renin extracts were assayed by mean blood pressure determinations from the cannulated femoral artery of the dog, using a standard intravenous dose of 0.5 g of kidney equivalent per kg of assay animal. Successive injections were made at half-hour intervals in order to avoid cumulative pressor effects as far as possible. Contrary to the findings of Pickering and Prinzmetal,[12] we have found no significant difference in the response to renin of unanesthetized dogs and dogs anesthetized with sodium barbital. We have, however, found that bilateral nephrectomy performed one to 3 hours previously definitely increases the dog's reactivity to renin. Thus, whereas only one-third of 30 non-nephrectomized dogs, equally divided between anesthetized and unanesthetized animals, proved to be satisfactory assay subjects, two-thirds of 60 dogs nephrectomized one to 3 hours previously were satisfactory. Moreover, the reactivity to renin before and after bilateral nephrectomy was studied in a group of 10 dogs. Each animal showed an increased response following removal of the kidneys. This is in accord with the report of an increased pressor response to renin obtained in dogs nephrectomized 2 to 3 days previously.[13] Unsatisfactory assay subjects were about equally divided into 2 groups. One group of animals gave no pressor response or practically none (less than 10 mm of Hg) with the foregoing or larger doses of renin. The other was composed of dogs which gave a pressor response to the first injection and none to subsequent injections, frequently with a failure of the blood pressure to return to its original level.

Splanchnic stimulation was performed on the peripheral end of the nerve on one side for a total of 1 to 4 hours in a group of dogs by means of a shielded electrode and a Harvard inductorium set at 6 cm, with 2 dry cells and an interrupter which permitted alternate minutes of tetanization in the primary circuit. The non-stimulated, control kidney was removed prior to the period of stimulation in some of the dogs. A hypotension of 60-70 mm of Hg was produced and maintained for 2 to 4 hours by hemorrhage in another group of dogs following removal of one kidney as control. Both the splanchnic stimulated and the hypotensive dogs were anesthetized with sodium barbital.

---

11 Grossman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 40.

12 Pickering, G. W., and Prinzmetal, M., *Clin. Sci.*, 1938, **3**, 211.

13 Merrill, A., Williams, J. R., Jr., and Harrison, T. R., *Am. J. Med. Sci.*, 1938, **196**, 18.

The renin content of one-half of a group of 24 normal dogs was such as to give an average pressor response for both kidneys of 21-30 mm of Hg in a dose of one-half g of kidney equivalent per kg of assay animal. The remaining dogs were about equally divided between dogs showing a renin content less than this median group and those with a renin content greater than the median. The maximum renin content observed (2 dogs) was about 3 times that of the median group, as judged by pressor effects of 61-70 mm of Hg. There was no correlation between these variations in the renin content and the blood pressure levels of the dogs. The renin contents of the 2 kidneys of the same dog were approximately equal in 19 of the animals (79%) but definite differences occurred in 5 of the dogs.

Unilateral stimulation of the splanchnic nerve (equally divided between right and left) in a group of 31 dogs produced no significant effect on the renin content of the stimulated as compared with the non-stimulated control kidney. Thus the renin contents of the 2 kidneys were approximately the same in 24 of the dogs (77%); in 4 instances the stimulated kidney showed a greater content than the non-stimulated control, and in 3 cases the reverse was true.

There was likewise no significant change in the renin content of the kidneys of the group of 6 dogs subjected to hypotension, when these kidneys were assayed against the opposite control kidneys. Thus, the renin contents of the 2 kidneys were approximately the same in all 6 dogs. Three of the hypotensive kidneys, however, showed an increased amount of depressor substances.

These results lend no support to the possibility that renin plays a rôle in the maintenance of normal blood pressure. Thus there was no correlation between the renin content of the kidneys of normal dogs and their blood pressure levels. The significance, if any, of the variations in the renin content of different dogs and, in some instances, of the differences between the 2 kidneys of the same dog, is not apparent at present. A decreased amount of renin in the kidneys of old rats has been reported,[14] but unfortunately the ages of our dogs were unknown. Prolonged stimulation of the splanchnic nerve did not alter the pressor activity of the corresponding kidney. Whatever the function of the nerve endings which penetrate the cells of the renal tubules may be, it is most likely not concerned with the formation or secretion of renin, if, indeed, the latter occurs. Lastly, the hypotension employed did not significantly alter the amount of renin. It must be emphasized, however, that these findings do not

14 Grossman, E. B., and Williams, J. R., Jr., *Arch. Int. Med.*, 1938, **62**, 799.

exclude the possibility that renin plays a rôle in the maintenance of normal blood pressure.

Conclusions. 1. Bilateral nephrectomy under the above conditions increases the sensitivity of the dog to the pressor action of renin administered intravenously. 2. The renin content of the kidneys of dogs bears no relation to the blood pressure level of the animals. 3. Differences in the renin concentration of the kidneys of dogs and even between the 2 kidneys of the same dog, occur. 4. Stimulation of the splanchnic nerve produces no significant change in the renin content of the dog's kidney. 5. Hypotension as induced above does not alter significantly the renin concentration of the kidney of the dog. 6. There is no evidence at present for the possible rôle of renin in the maintenance of normal blood pressure.

10403

Effect of Protein Alimentation on the Secondary Sex Ratio of Offspring of Rats.

F. HOELZEL, ESTHER DA COSTA AND A. J. CARLSON.

From the Department of Physiology, University of Chicago.

The sex-determining theory which Geddes and Thompson[1] and others attribute to Girou, namely, that the better nourished parent tends to determine the same sex in the offspring, was tested by mating rats fed diets adequate or high in protein (containing 30% to 50% casein) with rats fed diets low in protein (containing 7.5% to 12% casein, but otherwise similar to the high protein diets). Albino, hooded and hybrid (hooded-albino) rats were used. When increases in weight suggested pregnancy, the females were isolated and placed on a stock diet. The sex of the offspring was determined within 24 hours after birth. A preliminary study which lasted 8 months during which 49 litters were obtained yielded some data supporting Girou's theory but needing further confirmation. Accordingly a further study was undertaken which thus far involved 15 months during which 652 litters were obtained.

In our main study, only one male was used for mating with the females in each of 7 groups including from 10 to 17 individuals. Thus we obtained from 52 to 126 litters from each male within a year. Genetic variables were also reduced to a minimum by using

[1] Geddes and Thompson, *The Evolution of Sex*, Revised Ed., London, 1904.

the same females repeatedly for mating when possible but rotating them in random order between the different groups, excepting that albinos were mated only with albinos and hooded with hooded rats. Between Nov. 10, 1937, (the beginning of our main study) and Feb. 8, 1938, we obtained, from matings of low protein males with normal and high protein females, 51 litters (av. 8.2 young per litter) including 217 males and 203 females and yielding a sex ratio of 107 (males) : 100 (females). During the same period, we obtained, from matings of normal and high protein males with low protein females, 64 litters (av. 8.6 per litter) including 327 males and 226 females and yielding a sex ratio of 145 : 100. After this the diet of the latter groups was reversed. During periods of 67 to 71 days, respectively, we then obtained, from the same males, 56 litters (av. 8.1 per litter) including 218 males and 237 females and yielding a sex ratio of 92:100. This is a striking decrease in the male ratio as the normal seasonal (spring) effect is an increase in the male ratio. Further confirmation of the theory of Girou was obtained, in some cases, when the diet was again reversed later in the study but, in other cases, no changes were produced or the results became complicated by increased age, physical deterioration or sterility of the animals as a result of the prolonged use of inadequate diets.

We believe that divergent secondary sex ratios such as we obtained in rats are mainly due to a sex-differential resorption of embryos. The state of protein metabolism induced in the parent evidently also affects the germ cells and tends to determine the survival value of the same sex among the embryos. The survival of a larger percentage representing the better nourished sex might be due to the expected transmission of sex-linked characteristics. The results would also be explained if mammalian reproduction is a modification of parthenogenesis in which each sex tends to reproduce itself with survival of the fittest.

10404

Metabolism of Intraperitoneally Injected Serum Protein.*

T. Addis.

From the Medical Department of Stanford University Medical School, San Francisco.

Two groups of 10 albino rats with identical average body weights were selected. For 2 days they were given no food. On the morning and evening of each day 12 cc of 6.4% dextrose in 0.9% sodium chloride was injected into the peritoneal cavities of the controls while to the experimental group an injection of 12 cc of freshly separated serum obtained from normal rats was given. Both groups were anesthetized 48 hours after the first injection and 18 hours after the last. All the blood that could be obtained from the cut abdominal aorta while the heart was still beating was centrifuged at high speed for half an hour and the serum separated from the clot. The quantity thus drawn represents about 75% of the total blood volume. The liver, kidneys and heart were then removed, weighed and prepared for the protein determinations. The contents of the alimentary tract were removed after boiling in 0.5 M acetate buffer at pH 5. and the protein content of the tract determined with the carcass.[1] In the experimental group the abdominal cavities contained on an average nearly 5 cc of a fluid that had a somewhat higher protein concentration than the serum that had been injected. This fluid was collected and its protein is not included in the total protein given in Table I.

TABLE I.
Effect of Intraperitoneal Injection of Serum on Total Organ Protein.

	Control—Dextrose Injection		Experimental—Serum Injection	
	Wt of organ	Protein of organ	Wt of organ	Protein of organ
Liver	6.451	1.235	7.356	1.522
Kidneys	1.279	0.199	1.492	0.235
Heart	0.751	0.121	0.765	0.132
Serum	3.490	0.212	4.495	0.427
Clot	3.315	0.861	3.560	0.992
Carcass and Alimentary Tract	196.081	32.015	205.717	34.430
Total	211.367	34.643	223.385	37.738

* This work was aided by a grant from the Rockefeller Foundation.

[1] Addis, T., Poo, L. J., Lew, A., and Yuen, D. W., *J. Biol. Chem.*, 1936, **113**, 497.

Since the original body weights of the two groups were identical the quantities found after 48 hours may be directly compared. It is evident that in all the organs examined serum injection is associated with a higher protein content than is found after dextrose injection. But the only important question is whether this greater protein content is a part of the rat's protein or is only the serum protein that we had injected. Now it will be noted that the serum content of the experimental group is double the serum content of the control group. This is due partly to an increase in the quantity of serum in the serum-injected group but mainly to an increase in protein concentration from 6.1% in the controls to 9.5% in the experimentals. It is reasonable, therefore, to consider that this singular hyperproteinemia is a passive phenomenon and represents the circulation of the injected serum protein that has been absorbed but not yet assimilated. However, the quantity of this presumably unassimilated protein is only 0.215 g while the total increase in protein distributed through all the exsanguinated organs and tissues of the serum-injected group is 3.095 g. In addition it must be remembered that the total amount of protein injected in the form of serum (after subtraction of what was found unabsorbed in the abdominal cavity and the small amount excreted in the urine), was only 2.417 g. The differences we observe cannot well be explained by a simple retention of unaltered serum protein. They are explicable on the assumption that the injected protein was metabolized. They thus extend the original demonstration of Holman, Mahoney and Whipple[2] that, in the dog, intravenously injected protein is utilized. On the other hand, the hypothesis advanced by Howland and Hawkins[3] that the injected protein is not broken down into amino-acids but is incorporated into the body proteins in the form of large aggregations of amino-acids has been rendered untenable by the work of Bergmann,[4] who has shown that each protein has its own individual periodicity in the arrangement of its amino-acids and by the work of Schoenheimer, *et al.*,[5] who have found that even indispensable amino-acids undergo intramolecular rearrangement before they are finally incorporated within the pattern of the amino-acid chains that constitute the proteins of the body. Howland and Hawkins were led to their view of the incomplete disintegration of the injected protein because

2 Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 269.

3 Howland, J. W., and Hawkins, W. B., *J. Biol. Chem.*, 1938, **123**, 99.

4 Bergmann, M., *Chem. Rev.*, 1938, **22**, 423.

5 Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, 1939, **127**, 333.

in phloridzinized dogs they did not find that protein injection was followed by the increased N_2 and sugar excretion that followed the taking of similar quantities of protein by mouth. But since Drury[6] has demonstrated the utilization of dextrose in phloridzinized dogs it is possible to suppose that the difference may be an indication of slow digestion of protein to amino-acids, not of no digestion at all. The preliminary hypothesis on which our future experiments will be based is that the injected protein is taken up by the reticulo-endothelial cells of the body and subjected to intra-cellular digestion to amino-acids.

10405 P

Utilization of Amino-Acids by *Clostridium botulinum*.*

C. E. CLIFTON.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California

Our knowledge of the metabolism of *Clostridium botulinum* is at present scanty. Wagner, Meyer and Dozier[1] analyzed the products of the action of *Cl. botulinum* on complex media but obtained little information as to the reactions by which the products had been formed. Knight,[2] summarizing the growth-requirements of *Cl. botulinum*, reports that the simplest medium which will support growth contains the amino-acids proline, glycine, leucine, alanine, lysine, and cystine, together probably with traces of tryptophan and of the "sporogenes growth factor." He suggests that *Cl. botulinum* may obtain its energy through the "Stickland reaction," a coupled oxido-reduction between pairs of amino-acids. Glycine and proline act as hydrogen acceptors while alanine and leucine act as hydrogen donators in the case of *Cl. sporogenes* (Stickland[3]). Therefore these amino-acids were tested to determine whether or not they are attacked directly as is true for *Cl. tetanomorphum* with various

[6] Drury, D. R., Bergmann, D. C., and Greeley, P. O., *Am. J. Physiol.*, 1939, **117**, 323.

* Aided in part by a grant from the Rockefeller Fluid Research Fund.

[1] Wagner, E., Meyer, K. F., and Dozier, C. C., *J. Bact.*, 1925, **10**, 321.

[2] Knight, B. C. J. G., *Bacterial Nutrition*, 1936, 117-120, His Majesty's Stationery Office, London.

[3] Stickland, L. H., *Biochem. J.*, 1934, **28**, 1746.

amino-acids (Woods and Clifton[4]) or by coupled reactions between pairs of amino-acids.

All the experiments reported below were carried out with washed suspensions of *Cl. botulinum,* Type B (E-44). 800 ml of plain broth plus 0.1% yeast extract were inoculated with 0.5 ml of a beef-brain culture of this organism and incubated for 20 hours at 37°C in a McIntosh and Fildes anaërobic jar. The culture was then centrifuged, the cells were washed in saline and finally suspended in phosphate buffer of pH 7.4. The suspension so prepared was immediately deaërated *in vacuo.* The usual Thunberg-tube method was employed for the detection of hydrogen donators and it was found that alanine and leucine were the only amino-acids of those essential for growth which reduced methylene blue readily. In the presence of glycine or proline, methylene blue was not reduced more rapidly than in the blank suspension alone. None of these amino-acids acted as hydrogen acceptors in the presence of reduced methylene blue, probably because the acceptor must lie on a more negative point on the O/R potential scale than methylene blue.

Direct reactions between pairs of amino acids were determined by measuring the ammonia production in the presence of glycine or proline. One ml of M/10 alanine or leucine, 1.0 ml of M/10 glycine or proline and 5 ml of the phosphate-buffer suspension of *Cl. botulinum* were placed in Thunberg tubes, which were then thoroughly evacuated. Appropriate controls were always included. After incubation for 6 hours at 37°C, ammonia was determined by distillation from 50% alcohol-borate buffer of pH 10 into standard sulfuric acid. Typical results obtained are shown in Table I.

The amounts of ammonia produced in the presence of a single amino-acid or the pair glycine plus proline are not appreciably greater than in the control suspension, but considerable amounts are produced from the pairs glycine or proline plus alanine or leucine. Apparently only one isomer of alanine is readily attacked, while proline does not appear to be deaminated.

TABLE I.

Substrate	M/10 NH_3 ml	Substrate glycine	M/10 NH_3 ml	Substrate proline	M/10 NH_3 ml
Control suspension	.17				
Glycine	.20	+ alanine	1.09	+ glycine	.25
d-l alanine	.20	+ leucine	1.18	+ alanine	.61
l-leucine	.25	+ proline	0.25	+ leucine	.98
l-proline	.23				

[4] Woods, D. D., and Clifton, C. E., *Biochem. J.,* 1937, **31**, 1774.

The reactions between alanine and glycine or proline have been studied in more detail and on a larger scale. Five ml of M/5 d-alanine plus 10 ml of M/5 glycine on incubation at 37°C in an atmosphere of nitrogen for 20 hours with the washed cells from 400 ml of medium gave on correction for the control 6.6 ml of M/10 CO_2, 12.8 ml of M/10 NH_3, and 10.5 ml of M/10 volatile acid (steam distillation). The volatile acid has been tentatively identified as acetic acid by micro Duclaux distillations. The results suggest that the main reaction between glycine and alanine may be represented as

$$2CH_2NH_2COOH + CH_3CHNH_2COOH + 2H_2O = 3CH_3COOH + CO_2 + 3NH_3$$

although the high value for CO_2 suggests that a side reaction may also be occurring.

In a similar experiment with 5 ml of M/10 d-alanine and 10 ml of M/10 l-proline, 5.0 ml of M/10 CO_2, 5.4 ml of M/10 NH_3, and 5.1 ml of M/10 volatile acid tentatively identified as acetic were recovered. Apparently proline is reduced without deamination, probably to δ-amino-n-valeric acid, as is true in the case of *Cl. sporogenes* (Stickland[5]). Experiments on a larger scale are in progress to isolate and identify by derivatives the reduction-product of l-proline and also the tentatively identified acetic acid. Preliminary results suggest that the reaction may be represented as

$$CH_3CHNH_2COOH + 2CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{\overset{\text{NH}}{|}}{CH} \cdot COOH + 2H_2O = CH_3COOH + CO_2 + NH_3 + 2CH_2NH_2 \cdot (CH_2)_3 \cdot COOH$$

These results also suggest that the oxidation of alanine by either glycine or proline may be represented as

$$CH_3CHNH_2 \cdot COOH - 4H + 2H_2O = CH_3 \cdot COOH + NH_3 + CO_2,$$

this oxidation probably taking place in 2 steps, pyruvic acid being the intermediate compound.

It has been shown in preliminary studies that *Cl. botulinum* does not obtain its energy by direct utilization of amino-acids as does *Cl. tetanomorphum*, but probably by a coupled oxido-reduction between pairs of different amino-acids in a manner similar to *Cl. sporogenes*.

[5] Stickland, L. H., *Biochem. J.*, 1934, **29**, 288.

10406 P

Correlative Differentiation of the Amphibian Epithelial Hypophysis.

A. B. Burch. (Introduced by J. F. Daniel.)

From the Department of Zoology, University of California, Berkeley.

In a previous paper[1] experiments were described which were designed to test the developmental capacity of the *pars buccalis* of the amphibian pituitary body, when prevented from making contact with the infundibular region of the brain. The experiments consisted in shifting the presumptive infundibular region of the late gastrula of *Hyla regilla* to a position posterior to that of the normal, thus forcing it out of reach of the *pars buccalis,* when the latter migrates inward to assume its definitive position at the anterior end of the notochord. At the time of operation, the anlagen of the two components of the pituitary body were separated by a distance equivalent to a quarter of the length of the embryo and it may be supposed that they exerted no influence over each other. This condition, however, does not prevail in post-neurula stages.

Tadpoles derived from gastrulæ upon which the operation had been performed exhibited the silvery-white condition which results from deficiency of secretion of the *pars intermedia* of the pituitary body. Histological examination of the "albino" specimens revealed that the infundibular recess had formed from the floor of the myelencephalon instead of from that of the diencephalon; the *pars buccalis* was situated in its normal position at the anterior end of the notochord and remained as a compact, darkly staining and comparatively undifferentiated body of cells, showing no trace of *pars intermedia* tissue.

From data available at the time, it was not possible to determine whether or not the isolated *partes buccales* of these "albino" larvae represented functional anterior lobes. Subsequent work, however, has illuminated this point.

The operation mentioned above was performed upon 52 gastrulæ of *Hyla regilla.* Of these, 47 developed into albino larvae which were cultured in sterilized spring water, fed upon boiled egg yolk, and maintained alive until the present time. For each of the experimental larvae, 4 control larvae from the same egg mass were kept under conditions identical with those of the experimental animals.

At 7 weeks after the operation, all of control specimens have com-

[1] Proc. Soc. Exp. Biol. and Med., 1938, **38**, 608.

pletely metamorphosed and have assumed the coloration and pigment pattern of the adult type. The experimental animals, however, remain as large silvery-white tadpoles, in which the limb buds are present as small translucent papillae, showing no differentiation of toes or other parts. Although no histological examinations have as yet been made, there can be no doubt but that the anterior lobe of the pituitary body, if present as such, failed to perform its normal thyrotropic activity.

Concerning the question of somatotropic activity of the "pituitary body" in these experimental animals, the data are only slightly less consistent. Most of the experimental larvae approximate their controls in size, and are larger than the control specimens when the latter have made any considerable progress toward metamorphosis. A few of the "albino" tadpoles are smaller than their control tadpoles but this seems attributable to effects of the operation other than the prevention of contact between the *pars buccalis* and *pars neuralis*. The lack of disparity in growth rate between the normal and experimental animals, however, is not considered as evidence of differentiation or even partial differentiation of the anterior lobe of the pituitary body, for similar lack of disparity in size between totally hypophysectomized tadpoles and their control specimens has been found to be the rule. No data are available as yet regarding the adrenocorticotropic activity of the isolated *pars buccalis*.

We may conclude that the *pars buccalis* is correlatively differentiating, being dependent for its development and function upon the inductive effect of contact with the infundibular region of the brain. A more detailed account of this work will be published later.

10407 P

Effect of Adrenalectomy and of NaCl on Intestinal Absorption of Dextrose.*

T. L. ALTHAUSEN, E. M. ANDERSON AND M. STOCKHOLM.

From the Department of Medicine and the Institute of Experimental Biology of the University of California, San Francisco and Berkeley.

Wilbrandt and Lengyel[1] in Verzar's laboratory found that adrenalectomy decreased the absorption of dextrose and of olive oil, and

* Supported by a grant from the Christine Breon Fund.

[1] Wilbrandt, W., and Lengyel, L., *Bioch. Z.*, 1933, **204**, 267.

showed that administration of cortical hormone restored it to normal. These workers performed their absorption experiments on rats between the third and sixth day after operation. Deuel, Hallman, Murray and Samuels[2] found no impairment of the absorption of dextrose in adrenalectomized rats that had been kept on Rubin-Krick salt mixture for 12 to 20 days postoperatively. Recently, v. Issekutz, Laszt and Verzar[3] reported diminished absorption of dextrose and of butterfat from isolated jejunal loops of adrenalectomized cats 3 to 5 days after operation.

We repeated and extended the work on rats mentioned above because the influence of the adrenal cortex on intestinal absorption of dextrose has an important bearing on the phosphorylation theory of preferential intestinal absorption. Six-months-old male and female rats were used for this study. In order to clear the intestine of food residue, our rats were fasted for 24 hours before the absorption experiment, but the adrenalectomized rats had access to sucrose for the first 8 hours of this period. A 20% solution of dextrose was then given by stomach tube in amounts approximately double the capacity for absorption. An hour later the animals were sacrificed in order to determine the amount of unabsorbed residue in the entire digestive tract by the method of Hanes.[4] The first experiment was performed with normal and adrenalectomized rats 5 days after operation. The second experiment was done with normal rats receiving 1% of sodium chloride in the drinking water for 7 days and with rats treated similarly for 5 and 20 days after adrenalectomy (Table I).

From our experiment with untreated rats it is seen that adrenalectomy markedly impaired intestinal absorption of dextrose in both

TABLE I.

	Rats not receiving NaCl				Rats receiving NaCl					
	Normal		Adrenalectomized 5 days		Normal		Adrenalectomized 5 days		Adrenal ectomized 20 days	
		Dextrose absorbed*		Dextrose absorbed		Dextrose absorbed		Dextrose absorbed		Dextrose absorbed
Sex	No.	mg	No.	mg	No.	mg	No.	mg	No.	mg
M	8	130 ± 7†	7	84 ± 18	4	135 ± 8	5	146 ± 44	8	116 ± 33
F	6	166 ± 9	8	78 ± 26	—	—	6	142 ± 15	—	—

*Per 100 g of weight.
†Standard deviation.

2 Deuel, H. J., Jr., Hallman, L. F., Murray, S., and Samuels, L. T., *J. Biol. Chem.*, 1937, **119**, 607.

3 v. Issekutz, B., Jr., Laszt, L., and Verzar, F., *Pflügers Arch.*, 1938, **240**, 612.

4 Hanes, C. S., *Bioch. J.*, 1929, **23**, 99.

sexes. The degree of impairment is very similar to that occurring after thyroidectomy, as reported by Althausen and Stockholm.[5] Adrenalectomy, like thyroidectomy, nullified the normal difference[2, 5] in absorption of dextrose between sexes.

Our experiment with rats given sodium chloride shows that this substance did not alter the absorption of glucose in normal rats, but restored it to normal in adrenalectomized animals. This finding is of particular interest in view of the work of Anderson and Joseph,[6] who showed that administration of sodium chloride corrects the abnormalities of potassium and sodium excretion in adrenalectomized rats. Administration of sodium chloride after adrenalectomy did not raise the average rate of absorption in female rats above that in males. The present work reconciles the contradictory reports regarding the effect of adrenalectomy on intestinal absorption of dextrose.

Conclusions. Adrenalectomy markedly diminishes intestinal absorption of dextrose. Administration of sodium chloride after adrenalectomy restores the absorption of dextrose to normal.

10408 P

Electrolyte Excretion Studies in Rats Maintained on Low-Na and Low-K Diets.*

EVELYN ANDERSON AND MICHAEL JOSEPH.

From the Institute of Experimental Biology and the Department of Medicine of the University of California, Berkeley and San Francisco.

The rates of excretion of radioactive sodium and potassium have been studied in rats which were reared on the McCollum low-sodium and low-potassium diets.[1] The low-sodium diet was found by analysis to contain 0.6% sodium; the low-potassium diet, 0.4% potassium. The animals were maintained on these diets from the time of weaning until the excretion studies were made 5 to 6 months later.

5 Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1938, **123**, 577.

6 Anderson, E. M., and Joseph, M., PROC. SOC. EXP. BIOL. AND MED., in press.

* We wish to acknowledge the assistance from the Federal Works Progress Administration, Project No. 8877-A5, and also from the Christine Breon Fund.

1 Orent-Keiles, E., Robinson, A., and McCollum, E. V., *Am. J. Physiol.*, 1937, **119**, 651.

The sodium-deficient rats were definitely inferior in growth and in general appearance. Their average body weight at 6 months was 163 g as compared with the average body weight of the control animals on the regular stock diet which was 352 g. The potassium-deficient animals more closely resembled the control rats. Their average body weight at the same age was 362 g. Both groups of rats on the special diets were given distilled water in the drinking bottles. Unfortunately there were no control groups of animals fed the special low-sodium diet to which adequate sodium chloride was added, and the same may be said with reference to the low-potassium diet. However, since the only difference between these two diets was that the low-sodium diet contained potassium chloride and the low-potassium diet contained sodium chloride and the group on the low-potassium diet grew as normal animals, it appears permissible to assume that the defective growth in the low sodium diet was not due to any other deficiency than inadequate sodium.

The animals were taken off the special diets 24 hours before the radioactive isotopes were administered and were given Locke's solution made up with 6% glucose by stomach tube in amounts of 10 cc 3 times a day. The radioactive sodium and potassium were prepared by the method described by Hamilton.[2] The technic used in the excretion studies was similar to that described in a previous paper by the authors.[3] Each rat was given 1 cc of an isotonic solution of the radioactive isotope intraperitoneally. The excretion of this material was followed over a 48-hour period. During this period the Locke's solution with glucose was given by stomach tube in 10 cc doses 3 times a day. Some of the rats with the sodium deficiency were given adrenal cortical hormone (Upjohn), 1 cc twice daily, in the 24 hours preceding the administration of the radioactive isotopes and during the 48-hour excretion period.

The results of this study are recorded in Tables I and II. It will be seen that the rats suffering from sodium deficiency excreted the radioactive sodium more rapidly than normal animals and retained the radioactive potassium in greater amounts than normal. In the potassium-deficient rats the urinary excretion of the radioactive sodium was the same as in the normal rats; however, there was a retention of radioactive potassium.

The finding of an increased rate of excretion of radioactive sodium and a retention of radioactive potassium in rats which are suffering

2 Hamilton, J. G., *Ibid.*, 1938, **124**, 667.

3 Anderson, E. M., Joseph, M., Lawrence, J. H., PROC. SOC. EXP. BIOL. AND MED., in press.

TABLE I.
Effect of a Low-Sodium Diet on Urinary Excretion of Sodium^{24} and Potassium^{42}.

	Sodium^{24}			Potassium^{42}		
	No. animals	0-24 hr. %	0-48 hr %	No. animals	0-24 hr. %	0-48 hr %
Control group: Rats fed standard diet I	6	31.4 (27.2-35.1)	43.8 (40.5-49.0)	7	6.0 (4.9-7.5)	9.5 (8-11.5)
Exper. group I: Rats maintained on low-Na diet	4	33.2 (28.0-41.3)	55.2 (51.3-60.8)	6	3.1 2.6-4.4)	7.1 (6.3-7.7)
Exper. group II: Rats maintained on low-Na diet but given cortin	5	31.0 (25.7-36.7)	49.9 (43.1-55.1)	5	6.9 (5.6-8.7)	14.4 (14.8-16.3)

TABLE II.
Effect of a Low-Potassium Diet on Urinary Excretion of Sodium^{24} and Potassium^{42}.

	Sodium^{24}			Potassium^{42}		
	No. animals	0-24 hr. %	0-48 hr %	No. animals	0-24 hr. %	0-48 hr %
Control group: Rats fed standard diet I	6	31.4 (27.2-35.1)	43.8 (40.5-49.0)	7	6.0 (4.9-7.5)	9.5 (8-11.5)
Exper. group I: Rats fed low-K diet	5	31.7 (27.8-36.0)	46.7 (40.4-49.3)	5	2.8 (1.9-3.4)	6.3 (4.6-8.9)

from a deficiency of sodium but which have an adequate potassium intake, suggests that the mechanism for the control of potassium and sodium excretion has been seriously disturbed. This alteration in function resembles that seen in untreated adrenalectomized rats.[4] Furthermore, the giving of cortin to the sodium-deficient animals caused the excretion rate of sodium to return to normal, and to increase the rate of excretion of potassium over that of the normal. It is apparent that sodium-deficient animals are more sensitive to cortin than are normal rats. The retention of the radioactive potassium in the potassium-deficient rats might be interpreted as due to the need of the body for more potassium.

Summary. (1) Rats which have been reared on a low-sodium diet excrete radioactive sodium more rapidly than normal rats, and retain radioactive potassium in greater amounts than normal. In this respect they resemble untreated adrenalectomized rats. The giving of cortin corrected this disturbance. (2) Rats deficient in potassium excrete radioactive sodium at a normal rate but retain radioactive potassium.

10409 P

Urinary Excretion of Radioactive Na and K in Adrenalectomized Rats, With and Without Salt.* †

EVELYN ANDERSON AND MICHAEL JOSEPH.

From the Institute of Experimental Biology and the Department of Medicine of the University of California, Berkeley and San Francisco.

Radioactive sodium and potassium‡ have been administered to adrenalectomized rats and the rate of urinary excretion of these substances determined. The advantage of using "tagged atoms" for studying the metabolism of sodium and potassium is that it offers a rapid and highly accurate method for detecting in the body and in the excretions the identical atoms of sodium and potassium which have been administered.

4 Unpublished data by the authors.

* We wish to acknowledge the assistance from the Federal Works Progress Administration, Project No. 8877-A5, and also from the Christine Breon Fund.

† We wish to thank Dr. John H. Lawrence of the Radiation Laboratory for providing us with the radioactive isotopes used in this study.

‡ The preparation of the radioactive isotopes has been described previously by Hamilton (*Am. J. Physiol.*, 1938, **124**, 667).

The excretion studies on the radioactive sodium and potassium were carried out under identical conditions. For each isotope 3 groups of animals were used. Group I consisted of 6 adult male rats 8 months of age, which had been adrenalectomized 9 days previously and had been maintained on a standard diet, the animals having access also to tap water since operation. Group II consisted of 6 rats of the same age and sex which had been adrenalectomized 8 days previously and since then had been kept on the same standard diet but instead of tap water, were given 1.0% NaCl solution to drink. In the potassium studies only 4 rats were used in this group. The control group consisted of 6 unoperated rats for the sodium studies and 7 for the potassium studies. These animals were of the same sex and age and were maintained on standard diet and tap water. Twenty-four hours before the radioactive isotope was given, the animals were taken off their regular food and drink and were given by stomach tube Locke's solution fortified with 6% glucose in amounts of 10 cc each 3 times a day. One cc of an isotonic solution of the isotope in the form of NaCl or KCl was given to each rat intraperitoneally. The Locke's solution with glucose was continued in 10 cc amounts by stomach tube 3 times a day during the 48-hour observation period when the excretion of the radioactive isotope was being followed. The urine was collected in 24-hour samples for 2 days and the amount of the radioactive isotope which had been excreted was determined as follows: The urine was evaporated to dryness and the amount of radioactivity in the sample was measured by a Lauritsen electroscope.

The results of this study are shown in Table I. Only excretion figures for the entire 48-hour period are recorded, in order to simplify the table, since the first 24-hour period showed excretion figures comparable to the total 48-hour period. The excretion of the radioactive isotope is expressed in percent of the total amount administered. The total sodium and total potassium which the animals excreted in the 48-hour period were determined in 3 animals of each group given the radioactive isotope.

It will be seen that the adrenalectomized rats of Group I which had not been treated with NaCl postoperatively excreted almost 3 times as much sodium in the 48-hour period as the normal animals. This is in accord with previous observations by others. Furthermore, this group of rats excreted 60% of the total amount of the administered radioactive sodium as compared with 43.8% in the normal animals. On the other hand, the animals of Group II which had been treated with NaCl postoperatively showed excretion figures for sodium

TABLE I.
Urinary Excretion of Sodium^{24} and Potassium^{42} and of Total Sodium and Potassium in Adrenalectomized Rats.

	No. days post-op.	No. of animals	Na^{24} 48 hr %	Total Na* 48 hr mg	No. of animals	K^{42} 48 hr %	Total K* 48 hr mg
Control group: Normal rats	0	6	43.8 (40.5-49.0)	87	7	9.5 (8-11.5)	41
Exper. group I: Adrenalectomized rats given tap water to drink	9	6	60.0 (53.8-64.7)	232	6	5.0 (4.0-6.4)	15
Exper. group II: Adrenalectomized rats given 1% NaCl to drink	8	6	45.0 (38.3-55.9)	81	4	10.7 (8.8-11.5)	40

*In each instance, total sodium and potassium figures are averages secured from 3 animals only of the respective group.

which were practically the same as those for normal animals. The potassium excretion studies show that the adrenalectomized rats maintained on salt excrete potassium as the normal intact animal, while those not given salt have a diminished excretion of the total potassium and furthermore they retained a greater percentage of the administered radioactive potassium than did the normal animals.

Summary. (1) Radioactive isotopes of sodium and potassium furnish a new method of study of the physiology of the adrenal cortex. (2) Adrenalectomized rats, unsupported by salt treatment, have an increased total sodium excretion and increased rate of excretion of administered radioactive sodium when contrasted with normal animals; the reverse is true of potassium. (3) Simple access to a 1% solution of table salt enables adrenalectomized rats to handle sodium and potassium in a way resembling that of normal rats.

10410

I. A Murine Typhus Virus Isolated from a Patient in Peiping, China.*

WEI-T'UNG LIU AND HUEI-LAN CHUNG. (Introduced by C. N. Frazier.)

From the Department of Medicine, Peiping Union Medical College, Peiping.

Although Gajdos and Tchang[1, 2] and Wu and Zia[3] worked on several local orchitic strains of typhus fever virus, their studies were chiefly limited to observations on guinea pigs. In view of the fact that slight scrotal swelling may occur in from 2 to 22% of male guinea pigs infected with the "epidemic" or human virus,[4, 5] and that the latter virus and the "endemic" or murine virus are known to have a definite cross immunity in guinea pigs, we feel that before the local orchitic strains are identified with the murine virus, it would be of some importance to study the behavior of these strains in the

* The authors are indebted to Dr. Samuel H. Zia for supplying them with a Mexican typhus virus, and for his many valuable suggestions.

1 Gajdos, E., and Tchang, J., *Studies on Typhus Fever in China*, Catholic University Press, Peiping, 1933.

2 Gajdos, E., and Tchang, J., *Arch. Inst. Pasteur, Tunis*, 1934, **23**, 37.

3 Wu, C. J., and Zia, S. H., *Chinese Med. J.*, 1938, Suppl. II, 221.

4 Biraud, Y., and Deutschman, S., *League of Nations Epidem. Report*, 1936, **15**, 99.

5 Mooser, H., Varela, G., and Pilz, H., *J. Exp. Med.*, 1934, **59**, 137.

rat and mouse, especially since one of the strains studied by Gajdos and Tchang, which produced scrotal swelling in male guinea pigs, when later brought to Tunis, was found by Sparrow[6] to cause only an inapparent infection in rats, and by Durand and Hombourger[7] to be incapable of surviving beyond 3 passages in mice. This communication reports the results of a study on a local strain which we isolated in November, 1937, from the blood of a typhus fever patient, and which, we have found, possessed most, if not all, of the reported experimental characteristics of the murine virus in the guinea pig as well as in the rat and mouse.

Our virus has now been passed through guinea pigs for 27 generations. In transferring the virus, inoculations were made with brain, tunica or brain-tunica emulsions from these animals. Sixty-seven guinea pigs were used, 55 of which were males. Among the 25 guinea pigs employed during the first 10 generations, 21 were males. Six of these animals weighed between 200 and 300 g, and perhaps should not be expected to show any scrotal swelling. Ten of the 15 adult male guinea pigs exhibited marked or moderate scrotal swelling. However, since the 11th generation the scrotal swelling has disappeared. It reappeared only once in 12 animals of the 16th generation and was moderate in one and slight in the other. This disappearance of scrotal swelling on prolonged transfers in guinea pigs has also been the experience of Gajdos and Tchang, and of Wu and Zia working on local strains, and of Lépine[8] and Mooser[5] working on typical murine strains. In most of our guinea pigs showing no appreciable scrotal swelling, there were various degrees of congestion and exudation in the tunica vaginalis, from which typical Rickettsia bodies could usually be demonstrated, provided the animals were killed during the first few days of fever. In some of the animals killed on the 11th to the 14th day of fever, typhus nodules were either absent or scanty in the brains. The incubation period was relatively short, varying in the majority of our animals from 4 to 8 days. Two guinea pigs infected with our virus were found 6 weeks later to be completely immune against the Mexican virus, when inoculated with one-tenth and one-fifth of an infected brain respectively, whereas the control animals developed both fever and scrotal swelling.

The brain of a guinea pig of the 15th generation was inoculated intraperitoneally into 3 albino rats. Since then we have successfully

6 Sparrow, H., *Arch. Inst. Pasteur, Tunis,* 1935, **24**, 56.

7 Durand, P., and Hombourger, K., *Arch. Inst. Pasteur, Tunis,* 1935, **24**, 70.

8 Lépine, P., *Arch. Inst. Pasteur, Paris,* 1933, **51**, 290.

carried the virus from rat to rat for 12 generations.[9] Guinea pigs were inoculated with the brains of rats of alternate generations. All of the 12 guinea pigs used showed typical fever, and in 6 of them there was a return of slight and transient scrotal swelling. For serial transfers the rats were sacrificed 12 to 14 days after infection. We have found that the brains of rats 30 days, 40 days and 90 days after infection with our virus were still infectious on inoculation into guinea pigs, showing that the virus could survive in the rat for as long as 3 months.[10] The majority of the 30 rats used in this series showed a febrile reaction (38.1°-39.3°C) lasting from 1 to 13 days after an incubation period of from 3 to 8 days.[11, 12] Typical Rickettsia bodies could be found in moderate numbers in the tunica vaginalis and occasionally in small numbers in the peritoneum, when the rats were killed 5 or 6 days after infection by the intraperitoneal route. We have succeeded in producing a heavy infection of the peritoneal cavity in benzolized rats inoculated with a saline suspension of tunica vaginalis of a guinea pig infected with a Mexican virus,[13] but 3 similar attempts made with our own virus have all failed. However, it is to be noted that in the latter case we used brain as inoculum instead of tunica vaginalis inasmuch as this experiment was started after our virus had been passed through guinea pigs for 20 generations and no longer produced scrotal swelling in these animals. A Weil-Felix test was done on the sera of 10 rats killed from 5 to 11 days after infection; 4 showed a negative reaction at a titer of 1:10, 1 a positive reaction at 1:10; 2 at 1:20; 1 at 1:80, and 2 at 1:320.

The brain of a guinea pig of the 16th generation was inoculated intraperitoneally into a series of mice. Subsequently the virus was passed through 7 generations of these animals at intervals of 10 days. The brains of the mice of the 2nd and the 5th generations were found to be infectious on subcutaneous inoculation into guinea pigs. These animals developed typical fever and agglutinins for Weigl vaccine in their sera.[14] Furthermore, typical intracellular Rickettsia bodies could often be demonstrated, though not always, in smears from the tunica vaginalis and the peritoneum of the mice up to the 7th generation.[15] They could be found as early as 48 hours after infection by the intraperitoneal route, but in large

9 Nicolle, C., *Arch. Inst. Pasteur, Tunis,* 1933, **21**, 349.

10 Nicolle, C., and Laigret, J., *Arch. Inst. Pasteur, Tunis,* 1933, **21**, 357.

11 Mooser, H., *J. Inf. Dis.*, 1929, **44**, 186.

12 Maxcy, K. R., *U. S. Pub. Health Rep.*, 1929, **44**, 1935.

14 Laigret, J., and Jadin, J., *Arch. Inst. Pasteur, Tunis,* 1933, **21**, 381.

13 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649 and 865.

15 Okamato, Y., *Kitasato Arch. Exp. Med.*, 1937, **14**, 99 and 113.

numbers only after the 4th or 5th day. After the 9th day they became mostly extracellular and began to disappear.[15]

Summary. A typhus virus was recovered from the blood of a patient with typhus fever and was found to conform to the murine type in its behavior in the guinea pig, rat and mouse.

10411

II. Typhus Virus Isolated from Rats and Rat-fleas in Typhus Houses.*

WEI-T'UNG LIU AND HUEI-LAN CHUNG. (Introduced by C. N. Frazier.)

From the Department of Medicine, Peiping Union Medical College, Peiping.

In our previous communication,[1] a virus of the murine type isolated locally from the blood of a patient with typhus fever was described. Recently Wu and Zia[2] recovered a typhus fever virus from the pooled brain emulsion of 3 out of 139 rats trapped from different households in this city, thus verifying the assumption that the rat serves as a reservoir host for the disease in this locality. It appeared to us, that to complete the evidence of a rat-flea-man cycle of transmission, it would be necessary to recover typhus fever virus from rats and rat-fleas caught in the houses of typhus fever patients who were suspected, on clinical and epidemiological grounds, to be suffering from the murine variety of the disease. This communication reports the observations on 2 rat-strains and 3 flea-strains of typhus fever virus obtained in this manner.

In October, 1938, four typhus fever patients from widely scattered parts of the city were seen in this hospital. They were cleanly in their habits and gave no history of contact with the body louse. Their disease ran a very mild clinical course. In the homes of 2 of the patients whose Weil-Felix reaction titer exceeded 1:2560, there was evidence of heavy infestation with rats. These were trapped for study. One rat† was caught on October 19th in the house of the

* The authors are indebted to Dr. K. H. Pang for growing one of the rat-strains on Maitland's and Zinsser's tissue media.

1 Liu, W. T., and Chung, H. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 350.

2 Wu, C. J., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 163.

† Six other rats were caught in the same house but were not brought to us for study.

first patient‡ who was admitted on October 6th on the 9th day of the disease. It was killed with chloroform, and 3 fleas were collected from the body. The fleas (after being washed in 70% alcohol and rinsed in distilled water) and the brain of the rat, which was aseptically removed, were ground in sterile physiological saline and separately inoculated into the peritoneal cavity of 2 guinea pigs. After an incubation period of 11 days the guinea pig inoculated with the emulsion of rat brain developed fever (above 40°C) and scrotal swelling. It was killed on the second day of fever, and typical Rickettsia bodies were seen in smears prepared from scrapings of the tunica. From the tunica vaginalis of a guinea pig of the second generation Rickettsiae were also grown on Maitland's and Zinsser's tissue media.[3] The virus has now been passed through 5 generations of male guinea pigs. The infection is invariably manifested by fever after an incubation period of from 4 to 6 days and by marked scrotal swelling. A large number of Rickettsia bodies were found in the tunica vaginalis of all the animals examined. Four albino rats were inoculated with the virus. All developed a febrile reaction (38.2°-39.6°C), lasting from 3 to 6 days, after an incubation period of 3 or 4 days. The sera of 2 of these rats killed 10 days after infection, *i. e.,* 2 days after the subsidence of fever, agglutinated Proteus OX19 at a dilution of 1:320. The guinea pig inoculated with the 3 fleas also developed fever after an incubation period of 7 days. Scrotal swelling in this guinea pig was slight, but in animals of subsequent generations it became very pronounced. This flea virus has now been passed through 6 generations of guinea pigs.

In the house of the second patient§ who was admitted on October 18th on the fourth day of the disease, one rat was caught on November 5th. This rat harbored 5 fleas. The brain emulsion of the rat was inoculated intraperitoneally into a guinea pig which developed fever after an incubation period of 6 days. Although there was no scrotal swelling, the tunica vaginalis was congested and hemorrhagic. Rickettsia bodies were demonstrated in smears from this tissue. Four of the 5 fleas, after being washed in 70% alcohol

‡ When we heard of this patient, the temperature was already returning to normal. Therefore, no attempts were made to isolate the virus from the blood.

[3] Zinsser, H., Wei, H., and Fitzpatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

§ Five cc of blood were taken from this patient on the 7th day of the disease and inoculated intraperitoneally into a guinea pig which died 4 days later. Another guinea pig inoculated in the same manner with 5 cc of blood taken on the 12th day of illness did not develop the disease.

and rinsed in distilled water, were suspended in sterile physiological saline and inoculated into another guinea pig. After an incubation period of 9 days, it developed fever and scrotal swelling. Rickettsia bodies were demonstrated in the tunica vaginalis. The 5th flea was identified as *Xenopsylla cheopsis*. We have now carried both the rat-strain and the flea-strain through 6 generations of guinea pigs which have thus far retained a characteristic Neill-Mooser reaction. On November 12th another 2 rats, which harbored 2 and 3 fleas respectively, were caught in the same house. A guinea pig inoculated with the 5 fleas developed fever but without scrotal swelling after an incubation period of 5 days. On subsequent passages, however, slight scrotal swelling appeared in the inoculated animals, and typical Rickettsia bodies were found in the tunica vaginalis. This virus has been successfully passed through 6 generations of guinea pigs. Each of 2 guinea pigs was inoculated with the whole brain of one of the 2 rats, and observed for 3 weeks. They failed to show any fever. However, each of these 2 guinea pigs, when later inoculated intraperitoneally with one-third of a mixed emulsion of the brain and the tunica vaginalis of a guinea pig infected with the flea virus obtained from the rat trapped in the house of the first patient, developed fever for one day but showed no scrotal swelling. It seems obvious that they were partially immune as a result of the previous inapparent infection. The fact that the brains of these 2 rats were less heavily infected with typhus virus than the fleas recovered from them might mean that the latter had only recently migrated to these rats from a typhus-infected rat, presumably following its death, so that there was not enough time for the new hosts to develop a heavy infection. Another possibility is that, as a medium for growth and multiplication of the Rickettsiae in question, the rat-flea was actually better than the rat brain. A fourth rat caught on November 27th died shortly after it was brought to the laboratory. Four fleas were collected from this animal. Two of the fleas were crushed, smeared on 2 glass slides and stained with Zinsser's basic fuchsin solution. An enormous number of morphologically typical Rickettsia bodies, chiefly extracellular, were found in one of the smears. The other 2 fleas are being fixed for section.

Summary. Five strains of typhus virus were isolated from rats and their fleas caught in the houses of 2 typhus fever patients who were suspected, on clinical and epidemiological grounds, to be suffering from the murine variety of the disease.

10412

Serological Studies on Polysaccharides Derived from Diphtheroids.

Sam C. Wong and T. T'ung. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

Previous findings[1] suggest that polysaccharides prepared from various cultural types of *Corynebacterium diphtheriæ* are group-specific. It seemed of interest to determine whether polysaccharides of similar nature could be prepared from common diphtheroids.

Stock cultures of *C. hofmanni,* obtained from the London Type-Culture Collection, and *C. xerosis,* isolated from the conjunctiva of a patient with dacryocytitis in the Peiping Union Medical College Hospital, were employed for this study. Both organisms are non-pathogenic for either guinea pigs or Chinese hamsters and their staining and biochemical reactions are typical for these organisms with the exception that *C. xerosis* failed to ferment saccharose. The growing of the organisms and the method of preparing the polysaccharides were the same as those reported previously[1] except that only alkali was employed for hydrolysis. The yield of polysaccharide for both organisms was small, the amount being equal to 1 to 2% of the dried weight of organisms. Incidentally it might be stated that this figure is fairly representative of the yield of all polysaccharides prepared from the corynebacterial organisms thus far studied. The polysaccharides obtained from both organisms were white amorphous powders, readily soluble in saline in a concentration of 0.5%, gave strong Molisch reaction, exhibited none of the usual reactions for protein, and did not reduce Fehling's solution.

In serological tests the polysaccharide of *C. xerosis* was found to react equally with all sera prepared against the various cultural types of *C. diphtheriæ;* these included strains of intermediate, of gravis and of mitis. Precipitin titers (1:100,000) similar to those obtained with homologous polysaccharides were found. On the other hand, the polysaccharide of *C. hofmanni,* even in concentration of 1:1000, reacted weakly or not at all with the same sera.

Two rabbits were immunized by intravenous injections of living cultures of *C. xerosis,* the amount of each injection being the suspension from a 24-hour Loeffler-slant culture. Injections were given on 3 successive days followed by 4 days of rest. Rabbits were

[1] Wong, S. C., and T'ung, T., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 422.

bled before each series of injection and the resulting sera were tested with various polysaccharides derived from *C. diphtheriæ*. This might serve to detect any early appearance of type-specific antibodies. It was found, however, that precipitins began to appear after the second week of immunization and that there was no difference in the titers of the sera at any time when they were tested with the polysaccharides prepared from various cultural types. The maximal titer of 1:100,000 for all polysaccharides was reached at the end of the fifth week of immunization.

On the other hand, when rabbits were immunized with living cultures of *C. hofmanni* the sera did not react with heterologous polysaccharides, although a titer of 1:100,000 with the homologous polysaccharides was attained.

The finding of an antigenically distinct polysaccharide in *C. hofmanni* is not out of keeping with the existence of group-specific polysaccharide in *C. diphtheriæ*, especially since the proper classification of *hofmanni* in the genus of *Corynebacterium* is regarded as uncertain by some critical bacteriologists. On the other hand, the presence of a group-specific polysaccharide in *C. xerosis* and other diphtheroid organisms which we have examined during the past year strongly supports the thesis of others that diphtheroids may represent, according to Jungeblut,[2] "degraded" species of true *C.*

10413

Significance of Acetyl Group in Determining Antigenic Activity of Bacterial Polysaccharides.

SAM C. WONG AND T. J. KUROTCHKIN.

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

The acetyl group[1] has been suspected to be responsible for the antigenicity of the specific polysaccharide derived from Type I pneumococcus. Recent findings by Felton and Prescott,[2] however, seem to indicate that the acetyl group may not be of great importance in determining antigenicity. Because of this discrepancy, investiga-

2 Jungeblut, C. W., *Agents of Disease and Host Resistance*, 1935, p. 949.

diphtheriæ.

1 Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

2 Felton, L. D., and Prescott, B., *Bull. Johns Hopk. Hosp.*, 1936, **59**, 114.

tion of other antigenic bacterial polysaccharides appears to be in order.

Two types of polysaccharides, one prepared by acetic-acid hydrolysis and the other by alkali-hydrolysis of the whole organisms, were obtained from *E. coli,* non-capsulated; and *K. rhinoscleromatis,* both the capsulated and the non-capsulated forms. All polysaccharides prepared by acid-hydrolysis possessed immunological properties similar to those of fraction SI[3] of *K. rhinoscleromatis* previously reported, in that they were able to bring about the active sensitization of guinea pigs and the production of the usual antibodies in rabbits. The polysaccharides prepared by alkali-hydrolysis, on the other hand, lacked such properties. Apart from this antigenic difference the acid- and the alkali-prepared polysaccharides of *E. coli* and the capsulated form of *K. rhinoscleromatis* were respectively indistinguishable either by serological or by absorptive tests with homologous immune serum. The polysaccharides prepared from the non-capsulated form of *K. rhinoscleromatis* differed in the precipitin-reaction but not in absorptive tests.[4]

Each fraction was purified by dissolving in distilled water and re-precipitating with alcohol in the presence of a few drops of concentrated hydrochloric acid. The precipitate was washed 5 times with 95% alcohol after which it was dried over a waterbath. One such treatment, as shown by preliminary tests of a few of the polysaccharide preparations, was apparently sufficient to give a substance of constant acetyl value. The serological activity of each polysaccharide when tested against the homologous immune serum was unimpaired by one or several of such treatments. In addition to these polysaccharides, 2 other preparations were included. These were the purified polysaccharides of the capsulated form of *K. rhinoscleromatis* treated in the following manner: The acid-hydrolysis fraction was boiled in 0.5% potassium hydroxide and the alkali-hydrolysis one was boiled in 1% acetic acid, both for 30 minutes, after which each was precipitated in alcohol. Each of them after drying was purified once more by the method outlined above. Both the polysaccharides thus obtained were non-antigenic. The acetyl group was determined by the micro-method of Pregl and Solty[5] and the results are presented in Table I.

It was found that the acetyl group was present in every preparation, the difference between the antigenic and the non-antigenic

[3] Wong, S. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 107, 113.

[4] Wong, S. C., *Ibid.*, 1938, **38**, 111.

[5] Pregl and Solty, *Quantitative Organic Microanalysis,* 2nd edition, 1930.

TABLE I.
Acetyl Content of Bacterial Polysaccharides.

Organism	Acetic-acid hydrolysis method		KOH hydrolysis method	
	Before treatment %	After* treatment %	Before treatment %	After† treatment %
K. rhinoscleromatis (capsulated)	6.45	4.54	1.61	4.95
K. rhinoscleromatis (non-capsulated)	6.18	—	5.75	—
E. coli	10.90	—	3.94	—

— = not done.
*Treatment with alkali.
†Treatment with acetic acid.

polysaccharides being only one of quantity. In the case of *E. coli* the difference was comparatively large (6.96%) while in the non-capsulated form of *K. rhinoscleromatis* the difference was less than 0.5%. In general it may be stated that the antigenic polysaccharides contained more than 6% of the acetyl group while the non-antigenic ones contained less. From these considerations it may seem obvious that a qualitative estimation of the acetyl group can not be employed as an index of antigenicity in the polysaccharides studied.

Whether the acetyl group is responsible for antigenicity in these polysaccharides cannot be determined with certainty. The evidence, however, seems to indicate that the acetyl group may not be of great importance. First, the difference of the acetyl content between the antigenic and the non-antigenic polysaccharides prepared from the non-capsulated form of *K. rhinoscleromatis* is only 0.43%, an amount which in our opinion is too small to explain the marked difference in antigenic activity. Second, when the purified antigenic polysaccharides prepared from capsulated form of *K. rhinoscleromatis* was heated with alkali, there was a loss of only 1.91% of acetyl content, yet its antigenic activity was destroyed. Third, when the purified non-antigenic polysaccharide of the same organism was heated with acetic acid there was an increase of 3.34% in the acetyl content, but the antigenicity of the polysaccharide was not restored.

Conclusion. Evidence is presented which suggests that the acetyl group is probably not responsible for antigenic activities of the polysaccharides prepared by acetic-acid hydrolysis of *E. coli* and the capsulated and the non-capsulated forms of *K. rhinoscleromatis.*

We are grateful to Mr. C. Y. Chow of the Department of Biochemistry for the determination of the acetyl group.

10414 P

Isolation of *Listerella monocytogenes* from Infectious Mononucleosis.*

C. A. Pons and L. A. Julianelle.

From the Pathological Laboratory, Monmouth Memorial Hospital, Long Branch, New Jersey, and the Department of Ophthalmology, Washington University School of Medicine, St. Louis.

The clinical condition commonly designated in this country as infectious mononucleosis, but classified elsewhere as glandular fever or monocytic angina, remains undetermined in etiology despite the attempts undertaken to clarify its nature. The more definite reports on this subject disclose a single observation by Nyfeldt[1] which suggests the causative agent to be an organism of the Listerella group, subsequently named *Bact. monocytogenes hominis.* More recently, Bland[2] expressed the opinion that a protozoön of the species Toxoplasma might be causally related to the disease. His study indicates that the "toxoplasma of glandular fever" is immunologically inseparable from *T. cuniculi,* but differentiable from the latter by its absolute virulence for monkeys and its greater virulence for rabbits. Since the etiology of this disease is still obscure and even disputed, it seems important to place on record observations made in our laboratories on a patient with infectious mononucleosis.

The clinical history of the individual studied is characterized by the more or less typical manifestations of the disease, which ran a course of about 3 weeks. A white girl, 16 years of age, had undergone chills, fever, and general malaise for 5 days preceding entry into the Monmouth Memorial Hospital. Upon admission, she complained of a severe angina, and on physical examination she was found to have a temperature of 102°F, enlarged cervical and axillary glands, and a palpable spleen, with no other particular signs. The results of her urinalysis were well within normal limits, while her blood gave a hemoglobin value of 76%, with a red-cell count of 3,300,000 and a white-cell count of 11,000, consisting of 23% polymorphonuclear cells, 37% lymphocytes, and 40% monocytes. Several blood enumerations were made during her stay in the hospital, and these revealed a fairly uniform hemoglobin and red-cell count,

* Aided by a grant from the Commonwealth Fund of New York to Washington University.

1 Nyfeldt, A., *C. R. Soc. biol.,* 1929, **101**, 590.

2 Bland, J. O. W., *Brit. J. Exp. Path.,* 1931, **12**, 311.

while the white-cell count reached as high as 17,700, with polymorphonuclears as low as 12% and both lymphocytes and monocytes attaining abnormally high figures.

Serologically, her blood contained no agglutinins for *B. abortus,* or typhoid, or paratyphoid bacilli A and B, but did agglutinate sheep's red blood cells (heterophile test) to a titer of 1:128.

Thus it is seen that a diagnosis of infectious mononucleosis is justified by the clinical manifestations, the changes in blood elements, and the presence of heterophile antibody.

A single culture made from the throat revealed predominantly hemolytic streptococci and a small gram-positive rod-like organism which was unfortunately discarded at the time as probably adventitious. A blood culture taken during the fourth day of hospitalization (ninth day of the disease) yielded on the fourth day of incubation a small gram-positive rod resembling that seen in the throat-culture. On plating, the organism was found to form small colonies, incompletely hemolytic, and suggestive of *Listerella monocytogenes.*

The organism isolated from the blood proved to be fatal for rabbits, guinea pigs, and white mice, in each animal inducing the lymphocytic-monocytic changes seen not only in the patient, but similarly observed by Murray, Webb, and Swann[3] as a result of spontaneous infection in the rabbit due to *Bact. monocytogenes.* The culture was found to agglutinate to titer in homologous anti-monocytogenes serum. It was also demonstrated that upon instillation in the conjunctival sac of rabbits or guinea pigs the organism caused a purulent conjunctivitis later characterized by corneal vascularization. This latter reaction will be described in greater detail in a subsequent communication as possibly diagnostic of *Listerella monocytogenes.*

Since the morphology, the motility, and the biochemical and fermentative reactions also coincided with the corresponding attributes of *Listerella monocytogenes,* the organism cultivated from the patient's blood was ultimately classified as such. Before identification of the culture was made, the patient had been discharged as well, and blood taken 6 weeks after recovery showed no agglutinins either for the organism isolated or for sheep's red cells.

[3] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.,* 1926, **29**, 407.

10415 P

Identification of *Listerella monocytogenes.**

L. A. Julianelle and C. A. Pons.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, and the Pathological Laboratory, Monmouth Memorial Hospital, Long Branch, N. J.

That *Listerella monocytogenes* may cause a severe experimental conjunctivitis in several animal species was pointed out in an earlier report.[1] While, as will be seen, the ocular infection is both a curious and characteristic reaction, it was not particularly emphasized at the time as a potential diagnostic test, since the study was made with only 2 strains. Since that communication, however, the isolation of this organism from the blood of a patient with infectious mononucleosis furnished the initiative for determining the possible accuracy of the conjunctival reaction in the identification of *Listerella monocytogenes.* While other procedures, such as the stimulation of mononucleosis in susceptible animals, agglutination in specific antisera, and fermentative reactions, are also helpful for the purpose, the test to be suggested is much more readily accomplished, requires little preparation, and eliminates entirely the question of interpretation, so that it is recommended as a method of simple and accurate diagnosis. Eight strains have been collected from various animal and human sources, and all have been found to give typical reactions, so it is felt that the test may be of actual value. While the number of cultures studied is small, it must be remembered that the total number of strains of *Listerella monocytogenes* available at the present time is not much more than twice as great.

In conducting the test, young cultures (18-24 hours) are applied either by instillation of heavy suspensions into the conjunctival sac or by swabbing the everted conjunctiva. This may be done by running a moistened swab over the surface of a blood-agar culture and then transferring the organisms by light massage to the conjunctiva of the animal to be tested. While the reaction has been reproduced with equal readiness in the rabbit, guinea pig, and rat, it has been found more convenient to employ the rabbit. A distinct conjunctivitis becomes visible 1 to 5 days following the application of bacteria. The lids rapidly become edematous, chemotic, and aggluti-

* Aided by a grant from the Commonwealth Fund of New York to Washington University.

[1] Morris, M. C., and Julianelle, L. A., *Am. J. Ophth.*, 1935, **18**, 537.

nated. Upon forcing the lids apart, there is an expulsion of thick, heavy exudate, composed in great part of monocytes. The conjunctiva is acutely and intensely inflamed, sometimes with petechiæ on the bulbar conjunctiva. The cornea appears turbid, the turbidity completely covering the cornea within 24 to 36 hours. Then the cornea may appear roughened or pitted and blood vessels begin at the superior limbus and reach the pupillary margin. The acute reaction varies in individual animals from 5 to 10 days, when the infection begins to subside slowly, complete healing requiring 1, 2, or 3 weeks.

Histological examinations of enucleated eyes reveals, in general, edema of the bulbar conjunctiva, advancement of the conjunctiva over the cornea, migration of numerous monocytes with a small number of lymphocytes into the conjunctiva, and congestion of the capillaries, which in certain instances are undergoing proliferation of the endothelial lining. The cornea exhibits infiltration with monocytes primarily and lymphocytes secondarily, the infiltration being particularly pronounced between the anterior lamellæ of the *cornea propria*. The corneal corpuscles apparently are unaffected. Moreover, the cornea is invaded by small capillaries which extend to all its depths.

It is important to point out that a number of other organisms tested in a similar manner have no effect on the eye. Even subconjunctival injection, which frequently causes a conjunctivitis, has no effect on the cornea. With the numerous bacteria studied, moreover, corneal infection was induced only by direct intracorneal injection.

The ocular infection due to *Listerella monocytogenes* remains localized in the eye, the organism being apparently unable to penetrate the conjunctival barrier into the blood stream. While occasionally the blood elements undergo changes characteristic of the organism, in general it has not been possible to establish any correlation between the presence and severity of the ocular infection and hematological change.

10416 P

Immunological and Serological Reactions of *Listerella monocytogenes.**

L. A. Julianelle and C. A. Pons.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, and the Pathological Laboratory, Monmouth Memorial Hospital, Long Branch, N. J.

In order to identify by serological means a suspected strain of *Listerella monocytogenes* isolated from the blood of a patient with infectious mononucleosis, several strains of the organism† were obtained from different workers for the preparation of antisera. Since preliminary tests indicated that the strains were not all antigenically identical, the investigation was extended to include a possible classification of these organisms based on serological reactions. Antisera were accordingly prepared in rabbits with 6 of 8 strains collected for the study.

The different cultures were subsequently tested for agglutination in the antisera, which varied in titer from 1 :1260 to 1 :5120. It was found that 8 strains of *Listerella monocytogenes* were divided by the agglutinative reaction into 2 varieties or types. For convenience, it is proposed to designate the types, I and II. Type I appears to be composed of 2 rabbit strains and 2 human strains, while Type II consists of 3 strains from 3 different animals (*i. e.*, cow, sheep, goat), not including the rabbit, and 1 human strain. This bears out to a measure the results observed by Webb and Barber[5] that 2 rabbit and 2 human strains and 1 gerbille strain tested by them fell into one group, which was as far as they went. It also corroborates and

* Aided by a grant from the Commonwealth Fund of New York to Washington University.

† We are indebted to the following for the cultures employed in this study: Dr. A. B. Wadsworth of Albany, New York, for strains No. 204 and No. 205, isolated from rabbits by Murray[1]; Dr. C. V. Seastone for strains which he isolated from encephalitis of cattle (D82-N), sheep (1975), and goat (48)[2]; Dr. C. G. Burn for strain No. 128, which he isolated from human meningitis[3]; Dr. E. W. Schultz for strain A.T_2, which he isolated from human meningo-encephalitis[4].

[1] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, **29**, 407.

[2] Seastone, C. V., *J. Exp. Med.*, 1935, **62**, 203.

[3] Burn, C. G., *Am. J. Path.*, 1936, **12**, 341.

[4] Schultz, E. W., Terry, M. C., Brice, A. T., Jr., and Gebhardt, L. P., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 1021.

[5] Webb, R. A., and Barber, M., *J. Path. and Bact.*, 1937, **45**, 523.

amplifies the observations of Seastone,[2] who showed that while strains from various animal sources, exclusive of the rabbit, were serologically the same, they were different from a single rabbit strain which he studied. More recently, it was shown by Schultz, Terry, Brice, and Gebhardt[6] that 2 serological groups occurred among 11 different strains of *Listerella monocytogenes* derived from various animals, including man.

While the number of strains employed in this study is too small to indicate more than a possible eventuality, it is tempting to speculate whether it may not be that these types actually indicate the ultimate source of the organism—Type I, for example, being essentially derived from rodent animals (rabbit, gerbille[7]), and Type II from ruminant animals (sheep, cow, goat). If future work should confirm this hypothesis, then it may be predicated that human strains originate from some animal source, and the source in question may be surmised from the agglutinative type.

Preliminary experiments suggest that immunity to infection is broader than the type-differentiation demonstrable by agglutination. Thus, animals recovering from experimental conjunctivitis induced by *Listerella monocytogenes* become resistant to further infection following instillation of either the homologous or heterologous strain. In this connection, it is curious that while recovery from conjunctivitis insures resistance to conjunctival reinfection, intravenous immunization resulting in high agglutinin-titers (1:1260 to 1:5120), on the other hand, offers no similar protection. Conversely, the immunity localized in the conjunctiva is more frequently than not unaccompanied by circulating antibodies.

10417 P

Flocculation with Staphylococcal Toxin and Antitoxin.

S. Edward Sulkin. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis

Bronfenbrenner and Reichert[1, 2] found that antitoxin obtained by immunizing animals against toxic filtrates of 24-day cultures of *B.*

[6] Schultz, E. W., Terry, M. C., Brice, A. T., Jr., and Gebhardt, L. P., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 605.

[7] Pirie, J. H. H., *Pub. S. African Inst. Med. Research*, 1927, **3**, 163.

botulinus precipitated the filtrate while antitoxin prepared against toxic filtrates of 4-day cultures did not precipitate. They concluded that the precipitation in the Ramon test[3] may be influenced by the antibacterial antibody in the antitoxic serum. A similar observation was reported by the author[4] using the tetanal toxin-antitoxin system. It seemed interesting to see whether these findings might also influence the practical application of the Ramon test in the standardization of staphylococcal toxin and antitoxin.

In a preliminary experiment it was found that an appreciable amount of toxin was present in the 48-hour filtrate of a toxin-producing strain of staphylococcus cultivated in the broth described by Parker, Hopkins, and Gunther.[5] The cultures were incubated at 37°C in an atmosphere of 10% CO_2. A potent toxin was produced by this strain after 20 days' incubation at 37°C. White mice were killed almost immediately by the intravenous injection of 0.3 cc of undiluted filtrate. Rabbits were immunized with the formolized filtrates of 48-hour and 20-day cultures, respectively, as well as with filtrates of 48-hour and 20-day cultures of a cream-colored atoxic variant of the same strain of staphylococcus. Isolation of the atoxic variant was accomplished by repeated sub-culturing in lithium-chloride broth. As expected, the antisera prepared against the non-toxic filtrates were found to be entirely devoid of antitoxin as determined by the protection-test on mice, since even 0.5 cc of undiluted serum failed to neutralize the lethal toxin contained in 0.3 cc of a 6-day culture-filtrate.

Rane and Wyman,[6] instead of using weak toxins and relatively large amounts of antitoxin, obtained true flocculation with a strong toxin (hemolytic streptococcus) and relatively few units of antitoxin. They suggested that when large quantities of antitoxin were employed in the test "it is conceivable that a bacterial protein rather than a neutralized toxin-antitoxin mixture was precipitated." In order that this point be considered in the present set of experiments, a constant amount of antigen was mixed in the *in vitro* tests with varying amounts of serum covering a very wide range so that the zone of precipitation, if narrow, might not be missed.

---

[1] Bronfenbrenner, J., and Reichert, P., Proc. Soc. Exp. Biol. and Med., 1925, **22**, 391.

[2] Bronfenbrenner, J., and Reichert, P., *J. Exp. Med.*, 1926, **44**, 553.

[3] Ramon, G., *Compt. Rend. de la Soc. de Biol.*, 1922, **86**, 711; *Ann. de l'Institut Pasteur*, 1923, **37**, 1001.

[4] Sulkin, S. E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 555.

[5] Parker, J. T., Hopkins, J. G., and Gunther, A., Proc. Soc. Exp. Biol. and Med., 1925-1926, **23**, 344.

[6] Rane, L., and Wyman, L., *J. Immun.*, 1937, **32**, 321.

The sera containing antitoxin and those obtained against the non-toxic filtrates were used in the precipitative test with the following antigens: (1) Filtrates of 48-hour cultures of the toxic and atoxic variants; (2) filtrates of 20-day cultures of the toxic and atoxic variants; and (3) 20-day filtrates of tetanus toxin (control). *In vivo* neutralization tests were also carried out at the same time, and in Table I, the letter "N" indicates the neutral point as determined by this method. Although the antiserum obtained against the 48-hour filtrate of the toxic variant neutralized the toxin in the *in vivo* tests, no precipitation occurred in the presence of the various filtrates tested. Similarly, no precipitation occurred when the antiserum prepared against the 48-hour filtrate of the atoxic variant was combined with the respective antigens. On the other hand, copious precipitation occurred when the antitoxic serum prepared against the

TABLE I.

		Antigen (2.0 cc in each tube)				
	cc	Filtrate of 48-hr culture of toxic strain*	Filtrate of 48-hr culture of atoxic variant	Filtrate of 20-day culture of toxic strain*	Filtrate of 20-day culture of atoxic variant	*Control* 20-day filtrate tetanal toxin (20,000 MLD per cc)
Antiserum	.10	—	—	+	—	—
against	.09	—	—	++	+	—
20-day	.08	—	—	+++	+	—
filtrate	.07	—	—	+++	++	—
of toxic	.066	—	—	++(N)	++	—
variant	.06	—	—	++	+++	—
	.05	—	—	+	++	—
	.04	—(N)	—	+	+	—
	.03	—	—	++	+	—
	.02	—	—	—	—	—
Antiserum	.10	—	—	+	—	—
against	.09	—	—	++	+	—
20-day	.08	—	—	++	+	—
filtrate	.07	—	—	++	+	—
of atoxic	.06	—	—	++	++	—
variant	.05	—	—	+	++	—
	.04	—	—	+	++	—
	.03	—	—	—	++	—
	.02	—	—	—	+	—
	.01	—	—	—	+	—

*White mice were killed almost instantaneously by intravenous injection of 0.3 cc of undiluted 20-day filtrate, while 0.5 cc of the 48-hour filtrate was necessary to produce acute and fatal toxemia in mice.

(N) = Neutral point in protection test; +++ = copious precipitation; ++ = moderate precipitation; + = slight precipitation; — = no precipitation.

NOTE: Antisera against 48-hour toxic as well as those against 48-hour atoxic filtrates failed to precipitate any of the antigens and, therefore, are omitted from this table.

20-day formolized filtrate was mixed with either its homologous toxic filtrate or with the 20-day filtrate of the atoxic variant. Likewise, the antiserum prepared against the 20-day filtrate of the atoxic variant produced a wide zone of precipitation in the presence of both the homologous filtrate and the 20-day filtrate of the toxic strain.

The results of these experiments indicate that (1) the filtrates of the young cultures of the toxic variant are relatively free from bacterial protein and hence their antisera contain antitoxin and no detectable antibacterial antibody; (2) that the filtrates of the old cultures of the toxic variant contain bacterial protein in addition to the toxin, thereby stimulating the production of both antibacterial antibodies and antitoxins; (3) that the usefulness of the Ramon test in the *in vitro* standardization of staphylococcal toxin and antitoxin is limited by the fact that the flocculating power is not strictly parallel to toxicity but depends upon the presence of bacterial proteins in the antigen and antibacterial antibodies in the antitoxic serum.

10418 P

Auto-Injection of the Biliary Passages from the Gall Bladder in Rabbit.

JOHN AUER AND LLOYD D. SEAGER.

From the Department of Pharmacology, St. Louis University School of Medicine, St. Louis, Mo.

Contraction of the rabbit gall bladder may drive its contents back into the biliary passages if the bile papilla is tonically contracted or if the choledochus is clamped, and dyes previously injected into the gall bladder may then be demonstrated in the liver and arterial blood.

Experimental Procedure. 300 mg sodium barbital subcutaneously per kg animal; clamp choledochus; expose gall bladder and withdraw 1 cc of bile; through the same hypo needle kept *in situ* inject 0.5 cc of 5% sodium fluoresceinate solution; clamp the puncture in the gall bladder by means of a small narrow-jawed bulldog clamp; to contract the gall bladder, 0.5 cc per kg of a crude secretin preparation containing histamin was injected intravenously.

Within one or 2 minutes after the secretin injection the gall bladder usually becomes tense, decreases in volume and the choledochus and hepatic ducts appear as bulging, rounded, green cords; the choledochus may be so distended that it is palpable. In 15 minutes ap-

proximately some liver lobes may show an irregular yellowish-green mottling on their surfaces. This mottling is generally most marked in the left anterior and posterior lobes and is least in the caudate lobe. At no time was any dye visible in the lymphatics of the gall bladder or the liver hilus. If the animal is now bled to death from the abdominal aorta, the dye may be demonstrated in the serum and the relatively bloodless liver now reveals the dye more clearly than before. If the liver substance is scraped away, scraping from the free border of a lobe toward the hilus, yellow hepatic ducts can be easily demonstrated. These yellowish ducts may often extend practically to the border of the lobe; not all hepatic ducts in any one lobe exhibit the dye, however, and their distribution is irregular.

On the basis of these results it seems probable to us that the chain of conditions described above plays a rôle in producing or maintaining a cholangitis or hepatitis when it happens to a subject with an infected gall bladder. This supposition is strengthened by several observations in rabbit where contraction of the gall bladder occurred at a time when the bile papilla was tonically contracted, so that clamping of the choledochus was not necessary; in these instances the choledochus and bile ducts became markedly distended with the dye-bile solution.

10419

Bacterial Synthesis of Cocarboxylase.*

M. SILVERMAN AND C. H. WERKMAN.

From the Bacteriology Section, Industrial Science Research Institute, Iowa State College, Ames, Iowa.

The authors[1] have submitted evidence indicating that *Propionibacterium pentosaceum* can phosphorylate vitamin B_1 to its pyrophosphoric acid ester (cocarboxylase). This synthesis was carried out in the presence of liver-extract and hexosediphosphate. More recently, Ochoa and Peters[2] have shown that vitamin B_1, or suitable pyrimidine fractions, can in the presence of cocarboxylase, catalyze

* Appreciation is expressed to Merck and Company for synthetic crystalline cocarboxylase, to Winthrop Chemical Company for synthetic vitamin B_1, and to Anheuser-Busch for the bottom-yeast.

1 Silverman, M., and Werkman, C. H., *Enzymologia*, 1939, **5**, 385.

2 Ochoa, S., and Peters, R. A., *Biochem. J.*, 1938, **32**, 1501.

cleavage of pyruvic acid. They have also reported that hexosediphosphate in itself increases the rate of breakdown of pyruvic acid by washed yeast in the presence of cozymase and cocarboxylase. This knowledge has cast some doubt on the bacterial synthesis of cocarboxylase reported by us. At present we wish to report on the synthesis of cocarboxylase from vitamin B_1 by *P. pentosaceum* in the absence of any added liver-extract or hexosediphosphate.

Methods. Materials. Bacterial cells. *P. pentosaceum* was transferred from a yeast-extract-peptone broth to the basal medium of Tatum, Wood, and Peterson[3] containing in addition 0.005% cystine and 0.075% hydrolyzed casein. Three transfers were made in the basal medium at 24-hour intervals, the third into 300 cc of medium. The cells were harvested at the end of 72 hours' incubation at 30°C and washed twice in distilled water. The yield from 300 cc of medium amounted to about 0.4 cc of cell-paste. Aqueous extracts showed the presence of only 0.3-0.4 γ of vitamin B_1 per g of dried cells.

Atiozymase. One g of dried brewer's "bottom" yeast was washed rapidly 3 times (*cf.* Ochoa and Peters) in 50 cc M/10 Na_2KPO_4 at 30°C. It was then immediately washed in distilled water and suspended in 10 cc of M/15 phosphate buffer, pH 6.2. One cc was employed in each Warburg vessel in the assay of cocarboxylase.

Chemicals. Magnesium was used as $Mg\ Cl_2$ C.P.

Winthrop's Vitamin B_1 and synthetic cocarboxylase (Merck) were employed. The vitamin B_1 solution contained 10 γ per cc.

Synthesis. The following were placed in each of two 50 cc centrifuge cups: 0.2 cc cell paste + 1 cc H_2O + 10 γ vitamin B_1 + 1 cc PO_4 buffer M/15, pH 6.2.

The cups were incubated in air at 30°C with frequent shaking for 4 hours and then placed in boiling water for 5 minutes. The B_1 was added to the second cup at the time of boiling. The cells were then centrifuged from suspension and 1 cc of the supernate was assayed for the presence of cocarboxylase.

Assay. The cocarboxylase assays were carried out in simple Warburg vessels at 30°C under an atmosphere of air. Each assay vessel

TABLE I.

Activator	Supernate Cup 1	Supernate Cup 2	2.3 γ cocarboxylase	Control*
mm^3 CO_2 in 30 min.†	324	28	236	21

*No added cocarboxylase.
†Values corrected for endogenous CO_2.

[3] Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

contained 9 mg pyruvic acid in the side cup and 1 cc of atoizymase and 0.1 mg magnesium in the main vessel. The total volume was 2.3 cc.

Since the assay system was not previously saturated with vitamin B_1, the assay is not quantitative for cocarboxylase, but there is no doubt that synthesis did occur, for where the vitamin was not incubated with the bacterial cells, there is almost no catalysis (cup No. 2).

Additional evidence that vitamin B_1 is converted into cocarboxylase by suspensions of *P. pentosaceum* grown in vitamin B_1 deficient media, may be obtained by comparing the effects of vitamin B_1 and cocarboxylase in the anaërobic pyruvate metabolism of these suspensions. In Table II are presented the data in which equimolar quantities of vitamin B_1 hydrochloride and synthetic cocarboxylase were added to 1 cc portions of 1:20 (by volume) cell-suspensions in M/15 PO_4 buffer, pH 5.6, and tested for its activity on 9 mg of pyruvic acid, as sodium pyruvate, under nitrogen in simple Warburg manometers at 30°C. In cups 3 and 5 the activators were placed in the side arm with the pyruvic acid; in cups 2 and 4, the activators were added directly to the suspensions in the main vessel. The contents were held at room temperature for 15 minutes and then incubated for 30 minutes with agitation in the waterbath. At the end of this time, the substrate and suspensions were mixed and readings were taken.

In Table II we note that in case of cells incubated with the catalysts (cups 2 and 4), the rates of CO_2 production from pyruvic acid are similar. Where cocarboxylase has been added from the side cup (cup 5), a lag of 30 minutes occurs before these cells attain the rates of No. 2 and No. 4. However, where vitamin B_1 is added from the

TABLE II.

Comparison of Vitamin B_1 and Cocarboxylase as Stimulants in the Anaërobic Pyruvate Metabolism of *P. pentosaceum* grown in Vitamin B_1 Deficient Media.

Cup	1	2	3	4	5
Activator	None	0.25 γ vitamin B_1		0.34 γ cocarboxylase	
		Main vessel	Side cup	Main vessel	Side cup
Interval min.					
0-30	28	95	51	95	60
30-60	8	64	35	59	51
60-90	7	54	32	53	50
90-120	7	55	41	56	52
120-150	6	42	37	44	50
150-180	5	46	41	46	47

Results in mm^3 corrected for endogenous CO_2.

side cup (cup 3), there is a lag of fully 120 minutes before this cell-suspension reaches a rate comparable to the others. A reasonable explanation for the existence of this lag is that time is required for the conversion of the vitamin B_1 into cocarboxylase in which form it acts as the catalyst. In any case, however, if no previous incubation with the bacterial cells is permitted, cocarboxylase is a more effective stimulant than is vitamin B_1.

Summary. Evidence is presented that cell-suspensions of *P. pentosaceum* are capable of converting vitamin B_1 into cocarboxylase.

10420

Occurrence and Significance of Oxalacetic Acid in Plant Tissues.*

ORVILLE WYSS, R. H. BURRIS AND P. W. WILSON. (Introduced by E. G. Hastings.)

From the Department of Agricultural Bacteriology, University of Wisconsin, Madison.

The investigations of Szent-Györgyi,[1] Stare and Baumann,[2] and Krebs[3] have established the importance of the 4 carbon dicarboxylic acids in the respiration of animal tissues and several species of bacteria, but extension of their findings to plant tissues has not been made. Such extension might be naturally assumed since succinic, fumaric and malic acids, as well as the corresponding amino acid, aspartic, and its amide, asparagine, are well-known constituents of most plant species. One line of evidence for demonstration of this assumption would be the detection of oxalacetic acid in respiring plant tissues. In connection with our studies on the respiratory enzymes of the root nodules of leguminous plants, examination of these was made for the presence of oxalacetic acid, but none was found.

Both the colorimetric method of Szent-Györgyi and Straub[4] and the manometric method of Ostern[5] were used for detection of the oxalacetic acid. Fresh plant tissue was cooled to 0°C, ground in a Nixtamal mill and the sap expressed in a Carver hydraulic press.

* Herman Frasch Foundation in Agricultural Chemistry, Paper No. 188.

1 Szent-Györgyi, A., *Studies on Biological Oxidations and Some of Its Catalysts*, Budapest, 1937.

2 Stare, F. J., and Baumann, C. A., *Proc. Roy. Soc.* (London), 1936, **121**B, 338.

3 Krebs, H. A., *Biochem. J.*, 1937, **31**, 2095.

4 Straub, F., *Z. Physiol. Chem.*, 1936, **244**, 117.

5 Ostern, P., *Z. Physiol. Chem.*, 1933, **218**, 160.

The proteins were precipitated with tungstic acid. The manipulations were made as rapidly as possible, and all instruments were chilled before use in order to reduce decomposition of any oxalacetic acid which might be present. In the recovery tests the oxalacetic was added immediately to the expressed plant sap.

In the colorimetric method hydrazine forms a pyrazalone ring compound with oxalacetic acid which gives a deep yellow color on the addition of sodium nitrite and potassium hydroxide. Although this method is quite satisfactory for animal tissues, it was found unsuitable for detection of small quantities of oxalacetic acid in plant tissues. The data in Table I are typical of results obtained with a large number of tissues. Consideration of these data shows that the sap was free of oxalacetic acid.

TABLE I.
Estimation of Oxalacetic Acid in Young Pea Roots.

Treatment	Oxalacetic added, micrograms/ml	Color computed as oxalacetic, micrograms/ml	Recovery, micrograms/ml
Nitrite only	0	34	
All reagents	0	15	
	6.5	16	1
	13.0	34	19
	26.0	40	25
	65.0	83	68
	130.0	145	130
	320.0	313	298

Color measured by light absorption with 4200 Å filter; all values corrected for blank on sap alone, which was almost colorless. Plants used were actively fixing nitrogen.

With the addition of nitrite alone a yellow color develops which corresponds to more oxalacetic acid than is found with all 3 reagents. This color can not be distinguished from that formed by oxalacetic in presence of all reagents even with the selective filters of the Evelyn colorimeter. The decrease in apparent oxalacetic content on addition of all reagents probably arises through decomposition of part of the nitrite by the hydrazine. It follows that the "blank" due to these interfering substances cannot be estimated with any great degree of accuracy, and hence estimation of oxalacetic acid by the colorimetric method is not possible unless the quantity present is relatively large—order of 50 micrograms per ml.

As all attempts to remove the interfering substances by various precipitation, extraction and adsorption procedures were unsuccessful, the manometric method[5] was tested. With proper temperature control, this method proved satisfactory with plant tissues. As little as 25 micrograms per ml of added oxalacetic acid could be quantitatively recovered, and 10 micrograms per ml could be detected.

A large number of saps from the tops and roots of leguminous plants in various stages of growth were tested for oxalacetic acid by the manometric method, but all results were negative. These data are in agreement with the experience of Szent-Györgyi and his associates, who report that oxalacetic acid is detectable in respiring muscle tissue only after addition of a suitable precursor, as fumarate, and/or inhibitors (arsenite, hydrazine) which "fix" the acid by preventing its reduction. Both fumarate and malate were added to the saps and minced tissues of young pea plants with and without arsenite (M/100); analyses were made after incubation periods of 15 minutes to 5 hours, but oxalacetic acid was not detected. These negative results do not necessarily mean that the cycle proposed by Szent-Györgyi is not concerned with the respiration of the plants examined, only that efforts to obtain a certain type of supporting evidence have been unsuccessful. This failure is not too surprising since even in animal tissues which respire at a much higher rate than do most plant tissues, oxalacetic acid occurs only as a fleeting transitory intermediate whose detection is quite difficult.

In light of the foregoing discussion the recent reports by Virtanen and Laine[6] of finding from 500 to 1000 micrograms of oxalacetic acid per gram of wet tissue in the tops and roots of the pea plant are quite unexpected. Initially, these workers used the colorimetric method, but in a recent note[7] they state that since this method is unsatisfactory for colored portions of the plant, they have discarded it in favor of the manometric procedure.

It is suggested that the high content of oxalacetic acid found in the pea plants grown in Finland may be correlated with excretion of nitrogen by these plants. Although Virtanen and associates[6] consistently have obtained excretion of nitrogen by leguminous plants, repetition of their experiments at other stations, in general, has been unsuccessful. Our recently published evidence[8] indicates that differences in the environment at the various stations may be an important factor in determining whether or not excretion is obtained. Such differences should be reflected in biochemical variations in the plant which would control the excretion process. The accumulation of a compound such as oxalacetic acid which is usually regarded as a transient intermediate in the respiration cycle might well represent such a biochemical variation as has been postulated. This compound

[6] Virtanen, A. I., *Cattle Fodder and Human Nutrition*, Cambridge University Press, 1938.

[7] Virtanen, A. I., Laine, T., and Roine, P., *Suomen Kemistilehti*, 1938, **11**B, 25.

[8] Wilson, P. W., and Wyss, O., *Soil Sci. Soc. Proc.*, 1937, **2**, 289.

could be readily converted into aspartic acid which is the sole compound excreted.[8]

A second difference which has been noted in the plants grown in Finland and those at the Wisconsin Experiment Station is the relation of excised nodules to oxalacetic acid. Virtanen[6] reports that excised pea nodules supplied with oxalacetic acid will fix free nitrogen as rapidly as does the intact plant, but if no oxalacetic acid is supplied, fixation does not take place. We have repeated these experiments, but have never succeeded in securing fixation. In a typical experiment 100 mg of oxalacetic acid were added to 5 g of ground nodules from Torsdag peas which had been inoculated with the Finnish culture, *Rhizobium leguminosarum* HX and which were actively fixing nitrogen. Initially, the nitrogen content of quadruplicate samples was: 23.45, 23.40, 23.25, and 23.35 mg; after 24 hours' incubation the following values were obtained: 23.45, 23.40, 23.60, and 23.45 mg. Other experiments already have been reported for peas,[9] and similar results have been obtained with nodules from the soybean and cowpea. It should be noted, moreover, that not only do the nodules from pea plants grown at this station contain no oxalacetic acid, but that they decompose this compound very rapidly. Under the conditions of the nitrogen fixation experiments, 30 to 40% of the added oxalacetic acid was decomposed in 30 minutes, about 75% in one hour and almost all in 3 hours. If the nodules are not minced, decomposition is not so rapid, but in this case it is doubtful if much of the added acid actually diffuses into the nodules.

Test of the hypothesis proposed in this paper can best be made by workers at the several experiment stations who are conducting experiments on the excretion of nitrogen. If the plants are regularly examined for oxalacetic acid, it should be readily established whether or not the occurrence of large quantities of this compound in leguminous plants is correlated with the excretion phenomenon.

Summary. Because a yellow color is produced on the addition of the nitrite reagent alone, the colorimetric method of Szent-Györgyi and Straub was found unsuitable for the determination of oxalacetic acid in plant tissue, unless relatively large quantities are present. The manometric method of Ostern proved satisfactory, and though as little as 10 micrograms of added oxalacetic acid per ml of plant sap could be detected by the method, the presence of oxalacetic acid in leguminous plants actively fixing nitrogen could not be demonstrated.

It is suggested that the presence of oxalacetic acid in leguminous plants may be correlated with the phenomenon of nitrogen excretion.

9 Wilson, P. W., *Ergeb. Enzymforsch.*, 1939, **8**, 67.

10421 P

Nature of Fatty Acids of Acetone Insoluble (Phospholipid) Fraction of Serum.*

ARILD E. HANSEN.

From the Department of Pediatrics, University of Minnesota, Minneapolis.

Although relatively little is known concerning the exact nature of the fatty acids present in the acetone insoluble (phospholipid) fraction of the serum lipids, practically all methods used for their determination consider these fatty acids to have a constant structure. Whether the method is based on oxidative, titrimetric or gasometric analysis or is dependent upon the determination of the lipid phosphorus, nitrogen, or choline, in the majority of the procedures it is assumed that the fatty acids in the phospholipid molecule contain 18 carbon atoms.

However, Wilson and Hansen[1] using a micrograviметric technic for the determination of the fatty acids of the serum found the average molecular weight of the total fatty acids in human subjects to be in the neighborhood of 290. Channon and Collinson[2] working with enormous quantities of ox blood also found the average molecular weight of the total fatty acids of the serum to be higher than that of an 18-carbon atom fatty acid, their values varying from 290 to 311. Further proof that long chain fatty acids are present in the blood is furnished by the studies of Brown and Hansen,[3] also, Brown, Hansen, Burr and McQuarrie[4] in human subjects, and of Tängl[5] in oxen, who found that arachidonic acid, a 20 carbon atom fatty acid, is present in appreciable quantities in the blood serum.

In an attempt to follow up a previous finding of a low degree of unsaturation of the total fatty acids of the serum in infants with eczema, the author[6] experienced difficulty in finding a satisfactory

* Aided by grants from the Medical Graduate Research Fund of the University of Minnesota and from Mead Johnson & Company.

1 Wilson, Wm. R., and Hansen, Arild E., *J. Biol. Chem.*, 1935-6, **112**, 457.

2 Channon, H. J., and Collinson, G. A., *Biochem. J.*, 1929, **23**, 663.

3 Brown, Wm. R., and Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 113.

4 Brown, Wm. R., Hansen, Arild E., Burr, G. O., and McQuarrie, I., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 281; *J. Nutrition*, 1938, **6**, 511.

5 Tängl, H., *Biochem. Z.*, 1930, **226**, 180.

6 Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1198; *Am. J. Dis. Child.*, 1937, **53**, 933.

method for the determination of the phospholipid fatty acids of the serum. It was decided that the same fundamental principles used in the method of Wilson and Hansen[1] for determining the characteristics of the total fatty acids could be applied to the determination of the fatty acids in the phospholipid fraction. For this purpose the acetone insoluble fraction of the serum lipids was separated by precipitating with magnesium chloride according to Bloor's method.[7] The unsaponifiable portion of the phospholipid fraction was removed when the soaps were still dissolved in alcohol. After the fatty acids were liberated they were analyzed in essentially the same manner as described by Wilson and Hansen.[1] Although considerable difficulty was at first experienced in standardizing the method, subsequent findings have been consistent to the point where publication seems warranted. The details of the method will be described together with the findings in certain pathological conditions in a later publication. The data from 18 determinations on 14 essentially normal infants and children are presented in Table I. All specimens were obtained after a fast of 14 hours.

The acetone insoluble (phospholipid) fatty acids were found to comprise from 21 to 38% (average, 31%) of the total fatty acids of the serum when determined by this procedure. In each instance, excepting case 1, the iodine value of the phospholipid fatty acid was

TABLE I.

Values for the Acetone Insoluble (Phospholipid) Fatty Acids and Total Fatty Acids of the Serum with Their Respective Molecular Weights and Iodine Numbers as Determined on 14 Essentially Normal Infants and Children.

Case No.	Acetone Insoluble (Phospholipid) Fatty Acids			Total Fatty Acids		
	Mg%	M.W.	I.N.	Mg%	M.W.	I.N.
1	88	299	97	261	280	97
2	112	321	109	384	300	101
3	96	302	96	331	290	—
4	64	290	—	199	284	—
5	159	300	129	411	287	120
6	58	301	—	277	288	107
7	108	293	—	281	282	—
8	136	299	—	470	293	99
9	123	299	117	388	284	106
10	108	318	113	283	289	109
10	81	316	125	220	298	120
10	106	297	133	279	288	127
11	137	301	109	406	292	101
11	102	309	120	306	288	—
12	136	302	103	656	280	—
13	106	297	105	361	288	—
13	68	306	109	283	284	—
14	150	303	114	506	287	94

[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

higher than that of the corresponding total fatty acid. Of particular significance were the findings as regards the molecular weights of the phospholipid fatty acids, which were consistently higher than those of the total fatty acids. That the high molecular weights in the acetone insoluble fraction are not due to the presence of great quantities of arachidonic acid is indicated by the relatively low iodine numbers as compared to the great degree of unsaturation of arachidonic acid. If we can assume that the fatty acids in solution can be completely titrated and are stable, neither breaking down into shorter chains nor combining to form still longer chains, then it is necessary for us to look for other possible explanations for the high molecular weights encountered. The presence of such a substance as lignoceric acid, a 24 carbon atom saturated fatty acid, which is found in sphingomyelin extracted from certain tissues of the body, is a possibility worthy of consideration to account for the high molecular weights found in the fatty acids of the phospholipid fraction of the serum. The existence of these long chain fatty acids in the phospholipid fraction indicates the necessity of the recalculation of the factors employed for their determinaion in that these fatty acids are assumed to consist on the average of only 18 carbon atoms in most methods used.

Summary. This microgravimetric technic for the study of serum lipids in 14 normal infants and children disclosed that the acetone insoluble (phospholipid) fatty acids comprised about one-third of the total fatty acids. These fatty acids were more unsaturated and also had higher molecular weights than did the total fatty acids. Most methods used for the estimation of fatty acids of phospholipid fraction assume the presence of 18 carbon atoms. Our finding of high molecular weights indicates the necessity of revision of the calculations used in their determination.

10422 P

Blood Hormone Iodine and Iodide Reserve in Relation to Basal Metabolic Rate.*

J. F. McClendon and Carl O. Rice. (With the technical assistance of Ralph White and William Foster.)

From the University of Minnesota, Minneapolis.

Five women diagnosed as having toxic adenoma were given 2 grains of desiccated thyroid per day with the results on the basal metabolic rate shown in Table I.

TABLE I.

Age	Basic Metabolic Rate % Before Thyroid	After Thyroid	Days Treatment
52	+19	+34	14
29	+33	+22	10
38	+25	+27	50
41	+42	+55	41
40	+40	+25	60
Average 40	+31.8	+32.6	35

From this it is seen that the average basal metabolic rate was not appreciably increased. The same treatment was given to 11 patients with exophthalmic goiter and the results were as shown in Table II.

This shows that there is no increase in the average basal metabolic rate of exophthalmic goiter patients on feeding 2 grains of desiccated thyroid per day. This contained 0.2% of iodide; thus 2 grains

TABLE II.

Age	Sex	Basal MetabolicRate % Before Thyroid	After Thyroid	Days Treatment
50	Female	+ 19	+ 3	14
50	Male	+ 36	+47	21
25	Female	+ 85	+81	42
55	Male	+ 53	+43	16
31	Female	+ 36	+82	30
48	Male	+ 39	+42	24
31	Female	+101	+63	42
18	Female	+ 49	+35	37
47	Female	+ 61	+66	21
47	Female	+ 25	+25	14
57	Male	+ 66	+75	15
Average 41		+ 51.8	+51	25

* Assistance in analyses was furnished by the personnel of Works Progress Administration Official Project No. 665-3-71-69 and the Graduate School.

contain 0.26 mg of iodine whereas iodized salt contains 0.02% iodine, and the average daily intake of 10 g would contain 1.5 mg or nearly 6 times as much iodine as is present in the desiccated thyroid. Such a dose of iodized salt is not considered sufficient to lower the basal metabolic rate as much as 2 grains of thyroid would be expected to raise it. Therefore it seems probable that in the patients being fed thyroid there was, on an average, a lessening of the secretion equal to the dosage. At any rate, some chemical means of determining thyroid hormone in the blood seemed desirable.

We define *iodide reserve* as the iodine extractable from blood with methanol followed by acetone, and *hormone iodine* as the non-extractable iodine. The assumption that this non-extractable iodine is thyroid hormone iodine is supported by the fact that (statistically) the basal metabolic rate is directly dependent on the hormone iodine in the blood: since the micrograms of hormone iodine in 5 cc of blood $= 0.01 \times$ (the calories per square meter per hour —20). The remarkable statistical finding, in this region at least, on persons not receiving iodine medication, is that the iodide reserve is $0.01 \times (60 -$ calories per square meter per hour). We explain this by assuming that in this region the iodine intake averages a constant and that with an increase in basal metabolic rate there is an increased transformation of iodide reserve into hormone iodine on the one hand and an increased elimination of iodide in the sweat and urine on the other.

Although we have not shown in a specific case that the patient is in great danger on loss of the iodide reserve, it is true in general that patients in the class in which we find (statistically) a loss of the iodide reserve are severely toxic unless they receive iodide medication.

The fact that the blood "hormone iodine" varies directly with the basal metabolic rate indicates that it is an actual measure of thyroid hormone. Such a demonstration has not been made by previous workers. The injection of 1000 micrograms of thyroxine-iodine raises the B.M.R. about 1.8 calories per square meter per hour. The increase of 0.01 micrograms of hormone iodine in 5 cc of blood raises the B.M.R. 1 cal./sq. m./hour. If this were uniformly distributed in a 75 litre person, it would represent 150 micrograms (or 270 micrograms for 1.8 cal). Since this is only ⅓ the injected dose, it seems probable that the concentration in the muscles is 3 times that in the blood. This was found in an autopsy.

10423 P

Structure and Function of Placenta and Corpus Luteum in Viviparous Snakes.

HERMANN RAHN.* (Introduced by B. H. Willier.)

From the Biological Laboratory, Cold Spring Harbor, and the Department of Zoology, University of Rochester.

The discovery of primitive placentæ and well-defined corpora lutea in the viviparous snake *Storeria* by the writer has prompted a preliminary survey of these structures in other live-bearing snakes. Further, an attempt has been made to ascertain the function of the corpus luteum. Of special interest is the possibility of homologizing the structure and function of these organs with those of the mammal and of determining their rôle in reptilian gestation.

Although placentation in the lizards and snakes[1, 2, 3] and corpora lutea in lizards[4, 5] have been described for certain European and Australian forms, they are now recorded for the first time in our North American reptiles. In certain species of the genera *Storeria, Potamophis,* and *Thamnophis,* a yolk-sac placenta exists during the earlier stages of pregnancy; it is more developed in the first 2 of these genera. "Implantation" is brought about by a constriction of the extremely thin, transparent uterus between each embryo giving the gravid uterine horn the appearance of a linked sausage. On the ventral side of the uterus which encases the large yolk-sac, is an elliptical, creamy-white area which marks the extent of the yolk-sac placenta. In this region both uterine epithelium and the opposing ectodermal chorion of the foetus have given rise to very large columnar cells which cytologically suggest an active exchange of substances. The modified maternal and foetal tissues of this circumscribed area lie in close contact with each other and correspond to similar yolk-sac placentæ described by Weekes[3] for Australian reptiles. Beyond this area the chorio-allantoic and the uterine wall are highly vascularized. In the genus *Storeria* the uterine epithelium is partly eroded, allowing the maternal capillaries to make direct contact with the chorio-allantoic membrane. This may be regarded as a very primitive beginning of an allanto-placenta.

---

* John D. Jones Scholar at the Biological Laboratory, Cold Spring Harbor.

1 Giacomini, E., *Arch. Ital. Biol.,* 1891, **16**, 332.

2 Flynn, T. T., *Rec. Austr. Mus.,* 1923, **14**, 72.

3 Weekes, H. C., *Proc. Zool. Soc. London,* 1935, 625.

4 Hett, J., *Z. mikr. anat. Forsch.,* 1924, **1**, 41.

5 Weekes, H. C., *Proc. Linn. Soc. N. S. Wales,* 1934, **59**, 380.

Of interest in connection with the placentation and great vascularity of the pregnant uterus is the occurrence of corpora lutea of the ovary. So far they have been found in seven viviparous species of the genera *Storeria, Potamophis, Thamnophis,* and *Natrix.*[6] Their occurrence in 2 South American snakes has just been recorded.[7] An examination of the structure of the follicle shows that at ovulation the granulosa layer apparently becomes detached from the theca, but remains inside the collapsed follicle and soon proliferates and reestablishes its connection with the theca interna. The luteal tissue which is apparently derived entirely from the granulosa layer, fills almost completely the remaining follicular cavity. The theca interna displays a great activity during the early formation of the corpus luteum and supplies supporting fibers, capillaries and blood vessels. These penetrate into the luteal tissue and become very prominent toward the end of gestation. The completely differentiated organ is strikingly similar to that of the mammal. Its structure is maintained throughout most of pregnancy and slowly disintegrates after parturition. The period of gestation varies with the species from about 2 to 3 months and longer.

The finding of corpora lutea during gestation suggested this organ might be responsible for the maintenance of intrauterine embryos. Consequently a series of snakes *(Thamnophis* and *Natrix)* were castrated during early pregnancy. Seventeen were completely and 3 unilaterally castrated. Of those completely castrated 6 were injected with a mixture of progesterone and theelin. Autopsies at intervals up to 25 days after the operation in each series revealed living young. However, preliminary work during the previous summer showed that abortion followed complete castration in 2 out of 6 cases. These latter findings are in better agreement with those of Clausen,[8] who reports that after castration the embryos either die or are absorbed during early and middle pregnancy. In view of this controversial evidence, further work is needed before final conclusions can be drawn on the function of the corpus luteum in reptiles.

---

[6] Rahn, H., *Anat. Rec.*, 1938, **72**, Suppl., 55.

[7] Fraenkel, L., and Martin, T., *C. R. Soc. Biol.*, 1938, **127**, 466.

[8] Clausen, H. J., *Anat. Rec.*, 1935, **64**, Suppl., 88.

10424

Progesterone in Adrenalectomized Rats.

EDWARD L. SCHWABE AND FREDERICK E. EMERY.

From the Departments of Materia Medica and Physiology, University of Buffalo.

Two recent issues of this journal contained reports on the beneficial effect of progesterone in rats following adrenalectomy.[1, 2] Similar effects had been noted in ferrets,[3] in dogs,[4] and for related compounds in man.[5] This is not surprising since Rogoff had discussed it as early as 1927[6] and, more recently, large corpora lutea induced by pituitary implants were found to ameliorate the symptoms of adrenalectomy.[7, 8, 9] Until last year negative results with progesterone were common. Five of these have been reviewed by Gaunt and Hays[3] and our attempts to show the value of progesterone were so unsatisfactory that we did not mention them in discussing the rôle of corpora lutea.[9] We feel confident now that our failures with progesterone were due to inadequate dosage. Until recently the progesterone* we used contained 1/25 mg per cc; now it is available in 5 or even 10 mg per cc. As soon as we increased the dose per rat to one milligram the rats lived.

Female rats were used throughout these experiments. The adrenals were removed in 2 operations at 25 and 30 days of age respectively. Body weights were usually recorded each day. The adrenalectomized controls were litter mates of the injected rats. All animals were kept in a constant temperature room at 80°F and were fed Purina dog chow and water. The injected rats were closely watched for signs of weakness and in only 2 cases was it necessary to give more than the usual dose of 1 mg daily, and in these cases only 2 extra milligrams were needed. Since in both water and oil control groups the majority of rats had succumbed by the 13th day, we

1 Gaunt, Robert, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 319.

2 Green, R. R., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 83.

3 Gaunt, Robert, and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767.

4 Thorn, G. W., and Engle, L. L., *J. Exp. Med.*, 1938, **68**, 299.

5 Simpson, S. L., *Lancet*, 1938, **2**, 557.

6 Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1927, **79**, 508.

7 Marrian, F. G., and Butler, G. C., *Ann. Rev. Bio. Chem.*, 1937, **6**, 303.

8 Gaunt, Robert, *Symp. Quant. Biol.*, 1937, **5**, 395.

9 Emery, F. E., and Schwabe, E. L., *Endoc.*, 1936, **20**, 550.

*We are indebted to Dr. Erwin L. Schwenk of the Schering Corporation for his kindness in supplying us with generous amounts of Proluton, a crystalline, synthetic progesterone in sesame oil.

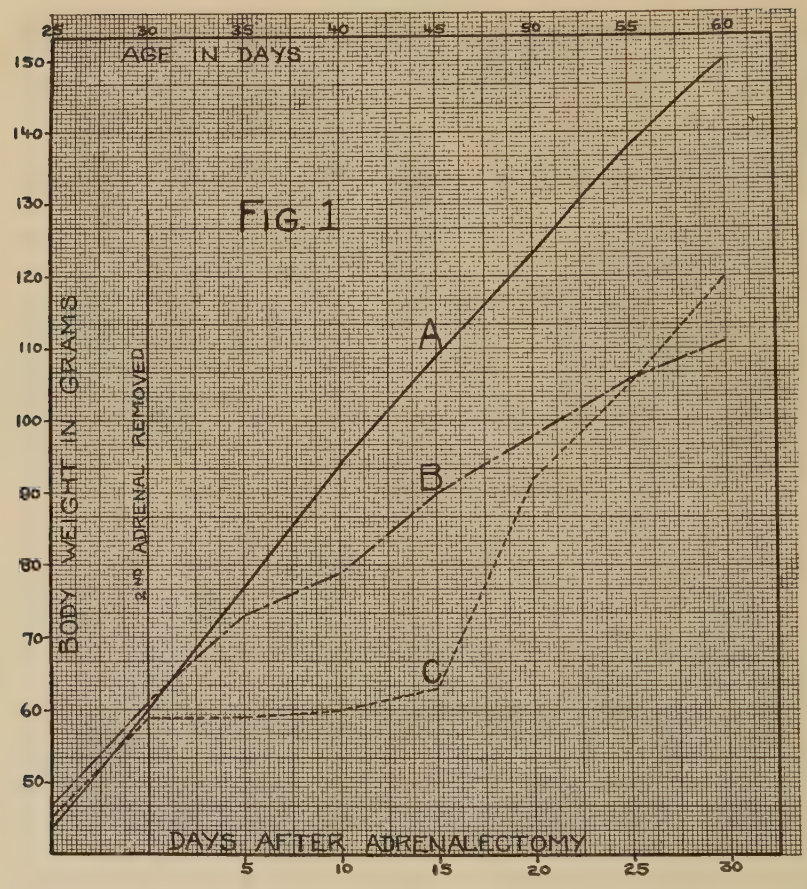


FIG. 1.

Mean body weights are shown for rats from 25 to 60 days of age. A—25 normal females. B—10 adrenalectomized females injected intramuscularly with 1 mg of progesterone daily for 13 days, then 1/5 mg for 7 days. C—30 adrenalectomized female controls.

decided to carry along the progesterone-treated animals with 1/5 mg daily from that date to the 20th day.

This procedure was sufficient to keep the weight curve of the treated animals about midway between the normal and adrenalectomized controls (Fig. 1). Larger amounts of progesterone would probably keep the weights equal to or even above normal, as was found for active corpora lutea induced by pituitary grafts.[9] All of the rats receiving progesterone lived and gained weight for 20 and more days (Fig. 1). After the injections were stopped, 4 rats died within 5 days and most of the others were not gaining in weight. The fact that hormones in oil are slowly absorbed probably helped to keep these rats going after progesterone was greatly reduced on the

13th day and also after it was discontinued. Estrin in oil is poorly absorbed when injected subcutaneously.[11] To avoid this all the injections were made intramuscularly.

The 30 adrenalectomized controls, 10 of which received ¼ cc of sesame oil daily, began to die on the seventh day after the second adrenal had been removed. The numbers alive 5, 10, 15, 20, and 25 days post-adrenalectomy were 30, 24, 11, 8, and 7 respectively. Removal of the adrenals in 2 stages was a factor in prolonging life. Also, different colonies of rats differ in the number of animals dying after adrenalectomy.[10] Our colony seems to be more resistant to the effects of adrenalectomy now than it was a few years ago; and our growth curve for normal rats is now greater (Fig. 1) than it was in 1936.[9]

Summary. Our results confirm previous reports that progesterone will keep adrenalectomized rats alive.[1, 2, 12] In our colony, after 2-stage adrenalectomy, doses of one milligram daily or less were usually adequate to keep young, newly-weaned, female rats alive and showing some gain in weight throughout the treatment period.

10425

Analysis of Present Methods of Collecting Tubercle Bacilli from Urine.*

HARRY A. FELDMAN AND JOHN H. HANKS. (Introduced by Leland W. Parr.)

From the Department of Bacteriology, Hygiene and Preventive Medicine, The George Washington University School of Medicine, Washington, D. C.

In a study on the concentration of tubercle bacilli from urine[1] two standard procedures were used as controls: Petroff's tannic-acid method† and direct centrifugation for one hour. Since the detailed

[10] Gaunt, Robert, *Am. J. Physiol.*, 1932, **103**, 494.

[11] Deanesly, R., and Parkes, A. S., *J. Physiol.*, 1933, **78**, 155.

[12] Fischer, A., and Engel, M., *Rev. franc. d'endocrin.*, 1938, **16**, 400.

* Aided by a grant from the U. S. Public Health Service.

[1] Hanks, J. H., and Feldman, H. A., to be published.

† *Petroff's Tannic Acid Method* (Trudeau Routine Laboratory Procedure):

1. Acidify urine with few drops of 30% acetic acid. To each 1000 cc of urine add 2 cc of 5% tannic acid. Store urine in ice-box 24 hours. Decant supernate and centrifuge sediment for 2 minutes at top speed. (In our experience, centrifugation for 5-10 minutes was required to pack the sediment.)

2. Decant supernate and dissolve sediment with N NaOH. Digest and add H_2O to fill the tube, centrifuge and decant the supernate again. Spread the sediment over clean slides.

3. Add a drop of N HCl and fix the smear with heat.

study of newer methods will not appear for some time, and since the results of one of the present technics are definitely improved by simplification, a brief analysis of this procedure is desirable.

Using Breed's counting method, the 2 procedures were analyzed quantitatively for their ability to collect human tubercle bacilli which had been added from "clump-free" suspensions to clear normal urine in predetermined numbers. The results were expressed as "concentration-factors" which indicated the increase in the average number of bacilli per microscopic field following concentration.

The concentration-factors were determined for direct centrifugation, for each of the steps outlined below for the Petroff procedure, and for a procedure which duplicated Petroff's, except for the omission of the tannic acid. Two methods—milk and HCl—for "fixing" the sediments to the glass slides were compared. The results shown in Fig. 1 are average values from 4 experiments in which different concentrations of bacilli were added to the urines. There were no exceptions to the general ratings as illustrated.

The results in the chart illustrate several useful facts: (a) The Petroff method appears to depend on the spontaneous precipitation of urates from acidified, chilled urine rather than on the use of tannic acid. It could be designated more properly as a "urate" concentration-method. (b) The first "urate" (or tannic acid) sediment produces a higher concentration-factor than is obtained by direct centrifugation. (c) The remainder of the Petroff procedure results

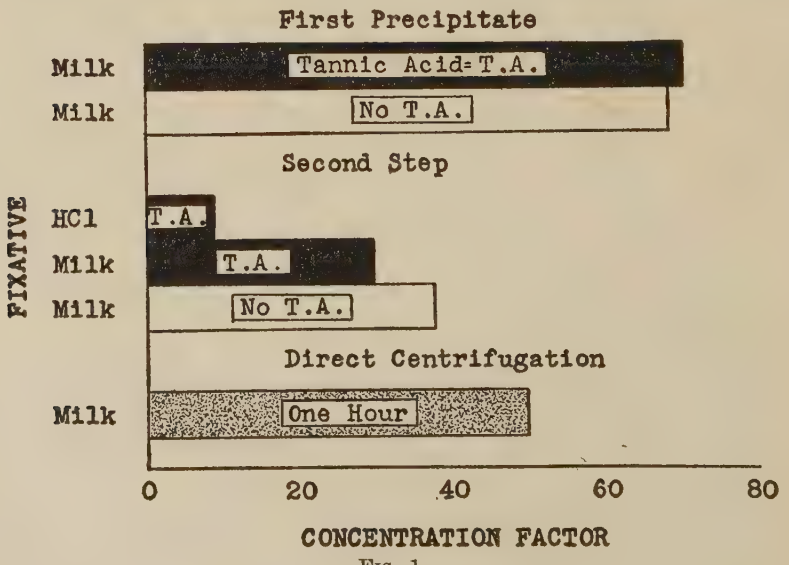


FIG. 1.

in a loss of the majority of the bacilli which were collected in the first step by precipitation. (d) Slide losses are reduced by the use of milk as a fixative.

These findings, in extension of our previous work,[2] demonstrate again that tubercle bacilli cannot be collected efficiently by direct centrifugation. Although the urate sediments in the first stage are extremely bulky, they contain more bacilli per unit volume than the very slight sediments obtained by direct centrifugation. For purposes of guinea pig inoculation or of cultivation, it should be noted that the total bacillary content of the urate sediments exceeds those from centrifugation by approximately one hundred times.

To wash, or to dissolve and recentrifuge a sediment, once collected by any method, results in a marked diminution of the numbers of bacilli collected.

10426

Attempted Transformation of Rabbit-Fibroma Virus into the Virus of Infectious Myxoma.*

JEAN SINCLAIR MECK AND ELLEN GRAY ACREE. (Introduced by Leland W. Parr.)

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, The George Washington University, Washington, D. C.

The following experiments were undertaken in an attempt to duplicate the results obtained by Berry and Dedrick,[1] who reported the transformation of fibroma-virus into that of infectious myxoma. The method of procedure followed throughout the experiments was identical with that of the original investigators[2] and consisted in inoculating domestic rabbits with a mixture of active fibroma-virus and heat-inactivated myxoma-virus.

The suspensions of viruses were prepared by grinding 10 to 20 g of virus-bearing tissue with alundum and 100 cc of Locke's solution. Five to 10 minutes before use, the suspension was centrifuged at about 1000rpm. Ten cc ampoules were filled completely with the

2 Hanks, J. H., Clark, H. F., and Feldman, H., *J. Lab. and Clin. Med.*, 1938, **23**, 736.

* The work reported in this paper was carried out under the direction of the late Earl B. McKinley.

1 Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, **31**, 50; **32**, 356.

2 Berry, G. P., personal communication.

suspension of myxoma-tissue, sealed in flame, and immersed for a total period of 35 minutes in waterbaths kept at 60°C, 75°C, and 90°C.

Each experiment, run in duplicate, consisted of 3 groups of rabbits; one group as a control to show that the fibroma-suspension alone produced "orthodox" lesions, one group as a control to prove that the myxoma suspension had been completely inactivated, and one group in which transformation was to take place.

In the group used as a control for the fibroma-suspension, 2 rabbits were inoculated in the following sites with a fresh 10 to 20% fibroma suspension diluted with an equal volume of Locke's solution:

A. Rt. side, head end, 1 cc.
B. " " tail " 1 "
C.
D. Left " 1 cc in each of 3 places.
E.
T. Rt. testicle, 1 cc.

In the group used to check inactivation of the myxoma-suspension, 6 rabbits were inoculated, 2 with the suspension inactivated at 60°C, 2 with the suspension inactivated at 75°C, and 2 with the suspension inactivated at 90°C. The following injections were made on each rabbit:

4 subcutaneously, ventral surface, 1 cc each.
1 intratesticularly, 1 cc.
1 intraäbdominally, 10 cc.

At intervals of 5 to 15 days, the inoculated testicles were removed, emulsified, and the suspension was injected into the testicles of a second group of 6 rabbits which in addition had received subcutaneous and intraäbdominal injections of the heat-inactivated material. This procedure was again repeated with a third set of rabbits.

In the group in which transformation was to take place, 6 rabbits were used. Two of these received the following injections:

A. Rt. side, head end, 1 cc of a 10 to 20% fibroma-suspension diluted with an equal volume of Locke's solution.
B. Rt. side, tail end, 1 cc of a 10 to 20% myxoma-suspension inactivated at 60°C, diluted with an equal volume of Locke's solution.
C. Left side, 3 cc of a 10 to 20% fibroma-suspension mixed with an equal
D. volume of 10 to 20% myxoma-suspension inactivated at 60°C.
E.
F. Rt. testicle, 1 cc of solution used in C, D, and E.

Two other rabbits received the same injections with the exception that the myxoma-suspension was inactivated at 75°C, and the last 2 received myxoma-suspension inactivated at 90°C. This experiment was carried out 3 separate times, and in each series the rabbits were observed for a period of at least one month. At no time has any of the animals in the control group for the inactivated myxoma-

suspension shown any evidence of myxoma. The fibroma-control groups have always been positive, and there has been no indication of myxoma in any of the animals in the "transformation" group.

We next tried a variation in pH, adjusting the inactivated myxoma to pH 9.4. Since earlier workers failed to get any transformation using myxoma inactivated at 90°C, the remaining experiments utilized only suspensions inactivated at 60°C and 75°C. The fibroma-control groups were positive; the groups inoculated only with inactivated myxoma-suspensions of pH 9.4 were negative, as were those in the so-called "transformation" group. Three animals of the myxoma-control group were later inoculated with a living suspension of myxoma and died within the customary period. A shift of pH to 3.8 was next tried and, as before, with negative results.

In a final set of experiments, to the inactivated myxoma-suspensions were added respectively, normal horse serum, trypsin, and a suspension of killed staphylococci. The normal horse serum was used undiluted. The trypsin-solution was made up with 1.4 g of trypsin per gram of myxoma-tissue and the mixture digested 48 hours. As a control normal rabbit tissue was also digested with trypsin. The staphylococcal suspension was heated for 35 minutes at 75°C.

These experiments were set up as before with the exception of the addition of the various substances named above to the inactivated myxoma-suspensions. Two rabbits were also inoculated with untreated myxoma-suspension inactivated at 60°C and 2 with untreated myxoma-suspension inactivated at 75°C. The fibroma-control group was positive, both the treated-inactivated and the untreated-inactivated-control groups were negative. Nor was there any indication of myxoma among the rabbits inoculated with the combined treated-inactivated myxoma- and fibroma-suspensions.

These results seem conclusively to show that the fibroma-virus (Shope) with which we were working could not be made to exhibit activity characteristic of the virus of myxoma by inoculating normal domestic rabbits with a mixture of active fibroma-virus and heat-inactivated myxoma-virus (Hyde-Moses). Hyde[3] also attempted to activate heated myxoma-virus by the addition of fresh fibroma-virus, but without success. In his words we are "at a loss to account for the difference in the results" between our findings and those of Berry and Dedrick,[1] which were confirmed by Hurst,[4] who states: "This remarkable observation was confirmed at the first time." It

[3] Hyde, R. R., *Am. J. Hyg.*, 1936, **24**, 217.

[4] Hurst, E. W., *Brit. J. Exp. Path.*, 1937, **18**, 23.

is not thought that the strain, age, or diet of the rabbits used can be the factor involved, for in the controls typical myxoma and fibroma developed. Fibroma-virus derives from Shope and in America myxoma-virus traces through Hyde or Rivers to Moses in South America. While originally identical it is likely there is a difference of many passages between the viruses used by Berry and Dedrick, Hyde, Hurst, and ourselves. It is possible that different viral strains or even the same strains kept in different laboratories may vary in their capacities to undergo alterations and that this may be the explanation for the contradictory findings on fibroma-myxoma inter-relationships.

10427

Decomposition of Sodium Mucate by *Aerobacter cloacae*.

A. A. HAJNA. (Introduced by Martin Frobisher, Jr.)

From the Bureau of Bacteriology, Maryland State Department of Health, Baltimore.

Brown, Duncan, and Henry[1] stated that the sodium salt of mucic acid is apparently not decomposed by *Aërobacter cloacæ*. Thus this singular property served to aid in the separation of this species of organism from the rest of the coliform group. In their study of the fermentation of salts of organic acids by various bacteria, a 1% peptone broth containing 1% of sodium mucate was used. To detect decomposition of sodium mucate, a solution consisting of 0.4 cc of glacial acetic acid and 0.6 cc saturated lead acetate was added to 5 cc of the culture. If the mucate was unaltered, a dense turbidity resulted which ultimately settled down in a small precipitate; but if decomposition of the salt had occurred, no precipitate followed upon the addition of the lead-acetate solution. However, they noted that on decomposition of mucate by other bacteria, sodium bicarbonate was one of the end-products formed. Thus, on the addition of lead acetate, a small precipitate of lead carbonate was formed. To overcome this disadvantage, a small amount of acetic acid (see above) was necessarily added to dissolve the lead carbonate and prevent false reactions. This is a tedious procedure.

In the author's study[2] on the decomposition of organic acids by

[1] Brown, H. C., Duncan, J. T., and Henry, T. A., *J. Hyg.*, 1924, **23**, 1.

[2] Hajna, A. A., *J. Bact.*, 1935, **29**, 253.

bacteria of the genus *Salmonella,* a synthetic medium with a pH indicator was found to be as satisfactory as peptone. If the organic acids were not decomposed, the medium remained clear and the indicator showed no evidence of a change in pH. If the test-substances were decomposed, this was evident by turbidity and a change in the color of the indicator.

The object of the present study was to determine whether *Aërobacter cloacæ* was able to decompose sodium mucate in Koser's[3] synthetic liquid medium, in place of detecting the change in a peptone medium.

The composition of the medium is as follows: Sodium mucate 0.3%, sodium ammonium phosphate 0.15%, monobasic potassium phosphate 0.1%, and magnesium sulphate 0.02%. The final hydrogen ion concentration is adjusted with sodium hydroxide to pH 7.0.

Tubes of mucate medium were inoculated with a loop (approximately 2 mm in diameter) with tryptone-broth cultures which were previously incubated at 37°C between 18 and 24 hours.

Sources of Cultures: Aërobacter cloacæ strains were isolated from the following sources: human feces 24, sewage 18, water supplies 12, crabmeat 2, oysters 24, and stock collection 8, making a total of 88. All cultures were purified by repeated serial transfers in Koser's liquid citrate medium and on Levine's eosin-methylene-blue agar plates. All *Aërobacter cloacæ* cultures were methyl-red negative; Voges-Proskauer positive; grew in citrate and uric acid synthetic media; failed to hydrolyze hippurate in a synthetic base medium (no growth); liquefied gelatin; produced no gas from glycerol at 37°C and from glucose, lactose and mannitol at 46°C. Indole and hydrogen sulphide were not produced.

Sixty of the 88 strains decomposed sodium mucate within 24 hours with the development of acid end-products as judged by the reaction of brom thymol blue. The remaining strains fermented it feebly. On further incubation at 37°C, all of the reactions reverted to the alkaline side and remained so for a period of at least 7 days with heavy growth in every instance. The reversion of the color of the indicator may indicate secondary decomposition resulting in the complete or nearly complete breakdown of sodium mucate into bicarbonate and other end-products.

Conclusion. Aërobacter cloacæ is able to decompose sodium mucate (in the synthetic liquid basic medium of Koser) and thus there is no apparent difference from the rest of the coliform group in ability to attack sodium mucate.

---

[3] Koser, S. A., *J. Bact.,* 1923, **8**, 493.

10428

Botulism and Picrotoxin.

A. H. Maloney.

From the Department of Pharmacology, Howard University School of Medicine, Washington, D. C.

Two phases are differentiated in botulinal intoxication: (1) a period of stimulation and (2) of respiratory depression. Experiments with picrotoxin point to reciprocal potentiation during the phase of botulinal stimulation and antagonism during the phase of depression.

The botulinal toxin (type A) in these experiments was prepared and supplied by the National Institute of Health in Washington. Its potency was expressed in terms of an intravenous mouse MLD, one unit being 0.00005 cc, the smallest amount that would kill a 17 to 20 g mouse when injected intravenously.

These experiments were performed on healthy stock rabbits ranging in weights from 1.45 to 1.85 kg.

Injected into the marginal ear vein 10,000 U (0.5 cc) of the toxin caused death (100%) within 13 hours average time. Three mg of picrotoxin intravenously administered is surely convulsant and frequently lethal; 2 mg is usually convulsant but non-lethal; while 1 mg is never lethal, rarely convulsant but regularly hyperstimulating to normal rabbits within this weight-range. However, when within the first hour following the injection of botulinal toxin, 3 mg, 2 mg, or 1 mg of picrotoxin was injected into the vein or even the muscle all the animals died within the first 2 hours with severe convulsions. But when an interval of 3 to 5 hours was allowed to elapse between the time of injection of toxin and picrotoxin with the picrotoxin divided into 0.1 mg doses each hour, subcutaneously, the life span was extended from an average of 13 (controls) to 23 hours. (Table I-a.)

Intraäbdominally, 20,000 U, 40,000 U, and 60,000 U, each of botulinal toxin was 100% lethal in 5 hours (average time). When administered by this route and in these amounts there began to appear signs of intoxication between the second and the third hour. When an interval of 3 hours was allowed to elapse and 0.1 mg of picrotoxin was given per hour, subcutaneously, the life span was increased from 5 hours (controls) to 15.9 hours. (Table I-b.)

Intravenously 20,000 U, 40,000 U, and 60,000 U, of botulinal toxin were in the aggregate 100% lethal in 1.8 hours, the signs of

TABLE I.

Controls		Treated (early)		Treated (delayed)	
Dose	Life Span, hr	Picrotoxin	Life Span, hr	Picrotoxin	Life Span, hr
a. Intravenous		(Within the first hour)		(3-5 hr after)	
(1) 10,000 U	13	3 mg—intramuscularly	1.1	0.1 mg/hr subcut.	20
(2) " "	14	2 " "	1.2	" " "	23
(3) " "	12	1 " "	1.4	" " "	22
(4) " "	13	1 "—intravenously	0.5	" " "	19
b. Intraäbdominal		(1 hr later)		(3 hr later)	
(1) 20,000 U	5.5	1 mg—intravenously	1.7	0.1 mg/hr subcut.	18.0
(2) 40,000 U	4.9	1 " "	0.9	" " "	15.3
(3) 60,000 U	4.8	1 " "	1.2	" " "	14.5
c. Intravenous		(1 hr later)		(1 hr later)	
(1) 20,000 U	2.4	1 mg—subcutaneously	5	0.2 mg/hr subcut.	7.0
(2) 40,000 U	1.6	1 " "	3.2	" " "	3.4
(3) 60,000 U	1.4	1 " "	1.3	" " "	5.4

intoxication being in evidence within the first 50 minutes. When 1 mg picrotoxin was administered subcutaneously one hour after the botulinal toxin the life span was extended to 3 hours. Better still, when the dose of picrotoxin was given as 0.2 mg each hour, subcutaneously, the life span was extended to 5.2 hours. (Table I-c.)

Small intravenous injections (10,000 U) and large intraäbdominal (20,000 U, 40,000 U, and 60,000 U) injections of botulinal toxin produce a slow toxemia characterized by restlessness, manifest discomfort, listlessness interrupted by "fits and starts", jerky irregular respiration, definite dyspnea, gasping, falling over, very slow respiration, and death.

Large intravenous doses (20,000 U, 40,000 U, and 60,000 U) produce rapid toxemia in which one observes hyperexcitability, manifest discomfort, dyspnea, gasping, falling over, and death in profound depression. In spite of the gradualness as against the rapidity of appearance of the symptoms in the 2 groups, both exhibit evidences of the 2 distinct phases, *viz.*, (1) stimulation, (2) depression. The second phase is ushered in by displays of manifest discomfort. The animal seems unable to remain quietly for any length of time in one place. Between intervals of quiet he jumps about as though the air in his environment is being shut off. This marks the period of transition.

If picrotoxin is given during the phase of stimulation its convulsant action is markedly increased so that a dose which would ordinarily be sub-threshold to convulsions may become a fatally convulsant dose. Here the 2 poisons definitely potentiate each other. If, however, picrotoxin is administered during the period of transition or early in the phase of depression they antagonize each other so that the impending embarrassment to the respiratory center is delayed.

The high mortality in botulinal poisoning is indicative of the limitation of our knowledge in handling this grave, but fortunately uncommon, condition. Bronfenbrenner and Weiss[1] discovered that certain depressants so extended the latent period of the poison that the employment of antitoxin could be postponed for hours provided that the animal was kept under the influence of ether anesthesia, luminal, nitrous oxide-oxygen or morphine. In acute poisoning delay is always desirable. Picrotoxin administered during the phase of stimulation in botulinal intoxication enhances the toxicity of the latter; but administered at intervals during the transition or the depressed phase in small amounts it antagonizes the toxic action of botulinus and delays the terminal event.

[1] Bronfenbrenner, J. J., and Weiss, H., *J. Exp. Med.*, 1924, **39**, 517.

10429

Penetration of Antiserum into the Central Nervous System of Monkeys Infected with Poliomyelitis.*

J. E. Kempf, W. J. Nungester and M. H. Soule.

From the Hygienic Laboratory, University of Michigan, Ann Arbor.

In the treatment of poliomyelitis, both convalescent and normal serum have been used, despite the fact that the virucidal property of such serum seldom exceeds a titer of 1:30. Since the virus in poliomyelitis is present and spreads within the CNS, antiserum to be effective must be able to penetrate readily into this area.

It is generally recognized that a physiological barrier prevents the entrance of blood proteins into the CNS tissues. Lennette and Campbell[1] have recently shown that the permeability of the blood brain barrier to the bromide ion was increased in experimental poliomyelitis in monkeys. They suggest the possible significance of such increased permeability on serum treatment of the disease. It seemed desirable to examine directly the permeability of the blood brain barrier to serum. Fox[2] has employed hemolysin to investigate the pulmonary permeability in normal animals, and in animals with sterile inflammation of the lungs.

With this work in view, monkeys previously inoculated intracranially with 0.5 to 1.0 cc of a 10% saline suspension of cord infected with poliomyelitis virus, Aycock strain, were injected after paralysis had been noted, with 10 cc of rabbit hemolysin serum against sheep red blood cells, having a titer of 1:40,000. After a definite time interval had elapsed (Table I), the animals were sacrificed by bleeding from the carotid artery, and in some instances, the residual blood was removed by perfusion with physiological saline solution. Spinal puncture was then performed, and specimens of spinal fluid withdrawn. Subsequently, various portions of the brain and spinal cord were removed, and approximately 0.5 g samples mixed with 3 cc of physiological saline solution and ground with glass beads in a shaking machine. The resulting suspensions were centrifuged and the supernatant fluid saved.

Serial dilutions were made of each of the tissue extracts, and also of the monkey's serum, spinal fluid, and the last sample of per-

* This work was aided by a grant from the Clara Ward Seabury Clinic for Infantile Paralysis.

1 Lennette, E. H., and Campbell, D. H., *Am. J. Dis. Child.*, 1938, **56**, 756.

2 Fox, J. P., *J. Immunol.*, 1936, **31**, 7.

TABLE I.
Penetration of Hemolysin into Tissues of Monkey with Poliomyelitis.

Monkey	Stage of disease when hemolysin was given	Hrs after injection before sacrificing	Titer of hemolysin in blood	Titer of hemolysin expressed as percent of that in corresponding blood sample*						
				Perfusion fluid	Spinal fluid	Lumbar cord	Cervical cord	Medulla	Site of inoculation of lesion	Lobe opposite inoculation site
1	Arm paresis, leg paresis	9	1:2560	12	1.5	1.5	1.5	1.5	1.5	1.5
2	Arms and right facial paralysis; paresis both legs	11.5	1:5120	12	0.2	1.7	0.8	3.4	1.7	1.7
3	Generalized weakness	24 (2 inj.)	1:2560	†	0.7	6.0	3.0	3.0	6.0	6.0
4	Quadriplegia	19	1:1280	†	13.8	6.0	6.0	6.0	6.0	6.0
5	Paresis both legs	38 (2 inj.)	1:2560	0.4	1.5	3.9	3.9	3.9	3.9	3.9

*If hemolysin were detected in no dilution of the tissue extract, the highest dilution found to be anticomplementary in the control test was assumed to contain hemolysin.

†Not done.

fusion fluid collected. These were tested for the presence of hemolysin in the usual manner, by the addition of normal guinea pig complement, 1% suspension of sheep red blood cells, followed by incubation in a waterbath at 37°C for one hour. As a control on the anticomplementary action of the tissue extracts, tests were carried out with similar dilutions to which a small amount of hemolysin (0.001 cc) had been added.

It was found that the titer of hemolysin in the blood was consistently high (Table I). Only a small percentage of this amount could be detected in the spinal fluid, although in the animal in which the longest time interval elapsed before sacrificing, there was a titer of 10% of that of the corresponding blood specimen. Considering only the lowest dilution shown not to be anticomplementary, it was found that less than 6% of the circulating hemolysin was present in any part of the CNS examined. In those instances where the tissue extracts gave a high titer of hemolysin the animals had not been perfused, and the values indicated probable contamination with blood.

Olitsky and Harford[3] found that when the virus of Eastern equine encephalomyelitis was injected intranasally or intracranially in guinea pigs, antiserum given intraperitoneally was without effect. However, if the virus were not localized in the CNS, but introduced intraperitoneally, similar amounts of serum protected against the disease.

On the basis of the aforementioned findings an attempt was made to account for the ineffectiveness of serum therapy in poliomyelitis. If we assume that 100 cc of normal or convalescent serum is injected intravenously into a 20 kilogram child with a blood volume of approximately 2000 cc, the concentration of antiserum in the blood becomes one-twentieth of the amount injected. Since the concentration in the spinal cord is less than 6% of that in the blood, it should be 6/100 of 1/20; or a dilution of 1:333 of the original antiserum. If the titer of the latter is 1:30, the amount in the spinal cord is only one-tenth of an adequate concentration.

Although it might be questioned whether it is the serum volume alone rather than the total volume of blood which dilutes the antiserum, we have found that calculations based on total blood volume agree with the resulting titer in the blood serum when hemolysin was injected in the monkey.

Since the potency of antiserum available for administration in poliomyelitis is low and since the amount administered to patients (seldom over 100 cc) is relatively 10 times less than that used in these experiments, it appears on a theoretical basis that antiserum

[3] Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 761.

administered intravenously would be without virucidal effect in the CNS.

These studies are being continued to include the effect of antiserum administered intrathecally.

Summary. Studies on the penetration of antiserum into the tissues of monkeys infected with poliomyelitis have shown that only a very small percent of circulating antibody is found in the spinal cord, thus suggesting little possible value in the use of antiserum in preventing the spread of poliomyelitis if such occurs other than by the blood stream.

10430

Effects of Insulin Treatment on the Cerebrospinal Fluids of Schizophrenics.

MONA SPIEGEL-ADOLF AND HERBERT FREED.

From the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University School of Medicine, and the Psychopathic Wards of the Philadelphia General Hospital, Philadelphia.

Changes of the non-electrolyte/electrolyte ratio (INE/IE) in cerebrospinal fluids were observed in cases of spontaneous convulsions[1] and in schizophrenics in which convulsions had been induced by metrazol treatment.[2] A marked parallelism was found between the values of INE/IE and the intensity of the convulsions. Evidence has been given that the increases of the ratio are due to an increase of the non-electrolytes (cleavage products of proteins and lipoids) rather than to a decrease of the electrolytes. The increase in cleavage products after convulsions was explained as due, at least partly, to changes of the cellular and vascular permeability.

In order to test some of these conclusions it seemed of interest to extend these investigations to the cerebrospinal fluids of schizophrenics treated with insulin. Although insulin has in such cases therapeutic effects similar to the ones of metrazol, the occurrence of convulsions is not paramount.[3] Moreover, observations have been reported indicating differences in the effects of the two methods of

1 Spiegel-Adolf, M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 92.

2 Spiegel-Adolf, M., and Freed, H., 94th Meet. Am. Psych. Assn., San Francisco, June, 1938. Will appear in *Confinia Neurol.*, 1939, **2**.

3 Cobb, S., *Arch. Int. Med.*, 1937, **60**, 1098.

treatment on brain metabolism,[4] with possible bearings upon the composition of the cerebrospinal fluids.

The method used in measuring the conductivity and interferometry values of the cerebrospinal fluids and in computing the INE/IE ratio was published previously.[1, 5]

The material on which our observations were based consisted in 73 samples of cerebrospinal fluids from 25 schizophrenics in various stages of insulin treatment. Previous observations[5] indicated that repeated tapping alone does not influence the INE/IE ratio. In all the cases, reports of the clinical symptomatology and in most of them data concerning the albumin and globulin content of the cerebrospinal fluids were available. Only a few sugar determinations have been made. The results were near the lower level of the average findings, confirming thus the results of Schretzmayr.[6]

Fifteen of our cases could be examined before the insulin treatment was started; 2 cases had undergone metrazol treatment before; in 8 cases, the measurements in the cerebrospinal fluids were made only after the patients had undergone insulin treatment. The average INE/IE ratios of these 3 groups were .27, .33, and .33 respectively. The first 2 figures check rather closely with similar figures reported in our studies upon the effect of metrazol treatment in schizophrenics; the third figure referring to possible effects of the insulin treatment will be considered later.

In 21 cases, complete reports as to the occurrence or non-occurrence of convulsions were available. Eleven cases of this group, which were observed before and during the whole course of insulin treatment never did show any convulsions. The average INE/IE values were .290 before the treatment and .292 after. Ten cases which all had shown convulsions of various intensity reached, after the insulin treatment, INE/IE values of .335 on the average.

Since, in the cases of epileptiform seizures, a certain parallelism could be observed between the severity of the convulsions and the height of the INE/IE ratio, an attempt was made to classify the cases which showed convulsions in the course of insulin treatment according to the number and the severity of these convulsions. If we order the material according to the number of convulsions, we find that 6 cases, which had between one and 4 convulsions, showed an average INE/IE value of .31. In 4 cases with 5-8 convulsions, the average INE/IE ratio was .38. If classified according to the

4 Himwich, H. E., Bowman, K. M., Farckas, T. F., and Orenstein, L. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 359.

5 Spiegel-Adolf, M., *Confinia Neurol.*, 1939, **2**, 1.

6 Schretzmayr, V., *Arch. f. Psych. u. Nervenkr.*, 1938, **108**, 680.

severity of the convulsions, in 5 cases the degree of the convulsions was recorded as moderate, the corresponding INE/IE value being .30. In the 5 other cases showing severe convulsions, an average INE/IE value of .376 was reached.

An attempt was made to correlate the changes in the INE/IE values to the clinical changes of the patients during the insulin treatment. In 8 of our cases, clinical improvements were reported while 16 showed relapses. The average INE/IE values for these two groups were .28 and .315 respectively. Although these figures seemed to indicate the possibility of prognostic value of the INE/IE ratios, it seemed necessary to ascertain how far the convulsions influenced the ratios in the improved cases as well as in the group with relapses. The material was divided into 4 groups: improvements without and with convulsions and relapse without and with convulsions. In the above sequence, the INE/IE ratios are .27, .33, .29, and .33 respectively. Only for the 2 groups without convulsions does there seem to exist a certain parallelism between the clinical findings and the changes in the INE/IE ratios. Nevertheless, it must be emphasized that even the value of .29, observed in cases showing relapses without convulsions, is somewhat below the ones observed in patients with organic lesions of the central nervous system.[7] In the cases with convulsions, the psychiatric findings and the INE/IE ratios seem to be independent of each other. The ratios in the cases with convulsions, however, correspond closely to the ones observed in patients with epileptic seizures.

Since metrazol is used in the treatment of schizophrenics in convulsion-producing doses, it was not possible in the metrazol group to decide how far the composition of the spinal fluid is influenced by the convulsions alone, and how far by the brain changes associated with the alteration in the psychic behavior of the patients. This question could be studied in the insulin group, since we have here changes in the mental picture with and without convulsions. The above analysis of these various groups seems to confirm the hypothesis that the increase observed in the INE/IE ratios is mainly due to the effect of convulsions.

Summary. The non-electrolyte/electrolyte ratios (INE/IE) were measured by combined conductivity and interferometry methods in cerebrospinal fluids of schizophrenics treated with insulin. A marked parallelism could be observed between increases of INE/IE and occurrence and frequency and severity of the convulsions. These findings confirm previous observations in epileptics and in schizophrenics under metrazol treatment.

[7] Spiegel-Adolf, M., *J. Nerv. and Ment. Dis.*, 1939, **89**, 311.

10431 P

Clinical, Electroencephalographic, and Biochemical Changes During Insulin Hypoglycemia.*

H. E. Himwich, J. P. Frostig, J. F. Fazekas, H. Hoagland and Z. Hadidian.

From the Departments of Physiology, Pharmacology, Neurology and Psychiatry, Albany Medical College, N. Y., and the Biological Laboratories, Clark University, Mass.

The present report is concerned with further investigation on the insulin treatment of schizophrenia. This work was done at the Harlem Valley State Hospital, Wingdale, New York, where patients with schizophrenia are receiving Sakel's insulin treatment. Ten observations were made on 5 patients. An attempt was made to correlate the clinical, biochemical, and electrical changes occurring as a result of insulin. It was hoped that such studies would establish a physiological basis for this treatment.

Clinical observations were made before insulin was injected. Brain waves were recorded at this time and samples of blood, drawn from the internal jugular vein and femoral artery were analyzed for oxygen[1] and glucose[2] in order to estimate cerebral metabolism. All these observations were repeated during various stages of hypoglycemia and after sugar was administered to terminate the coma.

The typical results here published were obtained on E.B. during hypoglycemia caused by a dose of insulin adequate to produce coma. The symptoms exhibited by the patient are presented and divided in 4 groups; the first including somnolence, perspiration, and hypotonia developed during the first 2 hours after the injection of insulin. Loss of consciousness, primitive movements, and forced grasping formed the second group. The third group is characterized by tonic and torsion spasms and occurred chiefly during the fourth hour. The final group in the fifth hour includes deep coma with depressed tendon reflexes and respiratory disturbances which necessitated termination of the hypoglycemia with sugar. Table I contains the results of the arterial-venous oxygen differences of the cerebral blood and values for arterial blood sugar, alpha frequency and delta index, as well as the time after the injection of insulin that these observations

* This work has been aided by a grant from the Child Neurology Research (Friedsam Foundation).

1 Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

2 Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

TABLE I.

Min. after insulin injection	Arterial-Venous O_2 Difference Vol.%	Blood Sugar mg%	Alpha Frequency per second	Delta-Index, cm
0	9.0	89	9.5	0.5
70	—	—	7.0	16.0
90	2.8	22	0.0	21.0
175	—	—	0.0	22.0
252	1.6	23	0.0	20.0
10 (After sugar)	4.5	45	7.0	—
30 " "	6.3	57	8.8	2.0

were made. The first group of symptoms appeared while the oxygen uptake fell from 9.0 volumes % to 2.8 volumes %. The second, third, and fourth groups developed in turn as oxygen uptake decreased further to 1.6 volumes %. The administration of sugar was followed by a rapid reversal of the symptoms and the blood picture. In view of the fact that there may be no significant change in cerebral blood flow during hypoglycemia uncomplicated by convulsions[3] it may be concluded that (a) there is a progressive decrease in cerebral metabolism as indicated by the diminution in the arterial-venous oxygen difference, (b) this decrease in oxygen consumption may be correlated with a lessening frequency and final disappearance of the alpha waves as well as an augmentation of the delta index, and (c) the progress of the symptoms through the 4 stages is also associated with an ever-deepening depression of cerebral metabolism. Thus a parallelism is revealed in the metabolic, electrical, and clinical changes resulting from the injection of insulin.

10432

An Indirect Method for Repeated Determinations of Blood Pressure of Rats.

J. T. DIAZ* AND SANFORD E. LEVY.† (Introduced by J. C. Burch.)

From the Departments of Obstetrics and Gynecology, and Surgery, Vanderbilt University School of Medicine, Nashville, Tenn.

A frequently used method for recording approximate mean blood pressures in rats is the insertion into the aorta, or other large vessels,

[3] Leibel, B. S., and Hall, G. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 894.

* This work has been aided by a grant to the Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

† National Research Fellow in the Medical Sciences.

of a needle which is connected to a mercury manometer. However, the animal must usually be sacrificed after such a procedure. An entirely satisfactory method which permits repeated determinations of blood pressure of rats has not been devised. Any improvement in the method now available is therefore desirable.

Recently described indirect methods are those of Griffith[1] and Bonsmann.[2] The use of the hypodermic needle and the optical manometer described by Hamilton, *et al.*,[3] is not simple technically, although it has many advantages. It was used by Woodbury and Hamilton[4] for recording the systolic and diastolic blood pressures in small animals. The method is not well adapted, however, for making repeated observations in small animals such as the rat, in which the blood vessels to be punctured must be exposed surgically at each determination.

In the present publication, an indirect method is described for making repeated determinations of blood pressure in rats. The results obtained with the indirect method are compared with pressures in the aorta recorded with the mercury manometer and needle.

Two methods of determination of blood pressure were used in these experiments. Direct determinations of the aortic pressure with a needle and mercury manometer were used to check the blood pressures obtained by the indirect method. The principle of the indirect method employed was the compression of the rat's tail by a cuff of a special design (Fig. 1). This cuff consists of a rigid outer cone of glass (approximately 3 cm in diameter and 7 cm in length) closed at each end with rubber stoppers. The stoppers each have a central hole. A tube of thin rubber (such as Penrose tubing) connects the holes in the stoppers. The pressure in the space between the outer glass tube and the inner rubber tube is regulated by a blood pressure bulb and mercury manometer. Anesthesia is induced by nembutal administered intraperitoneally. The rat's tail is placed through the central tube of thin rubber, which is of course open at both ends.

In order that the blood pressure be determined, the tail is adjusted so that a short length projects through the central tube. The pressure in the cuff is raised well above the systolic level. The tip of the tail is then snipped off with a pair of scissors and immediately immersed in a 0.2% solution of sodium citrate to avoid clotting. There is an initial flow of blood following the severing of the tail which diminishes until little or no blood oozes from the cut end.

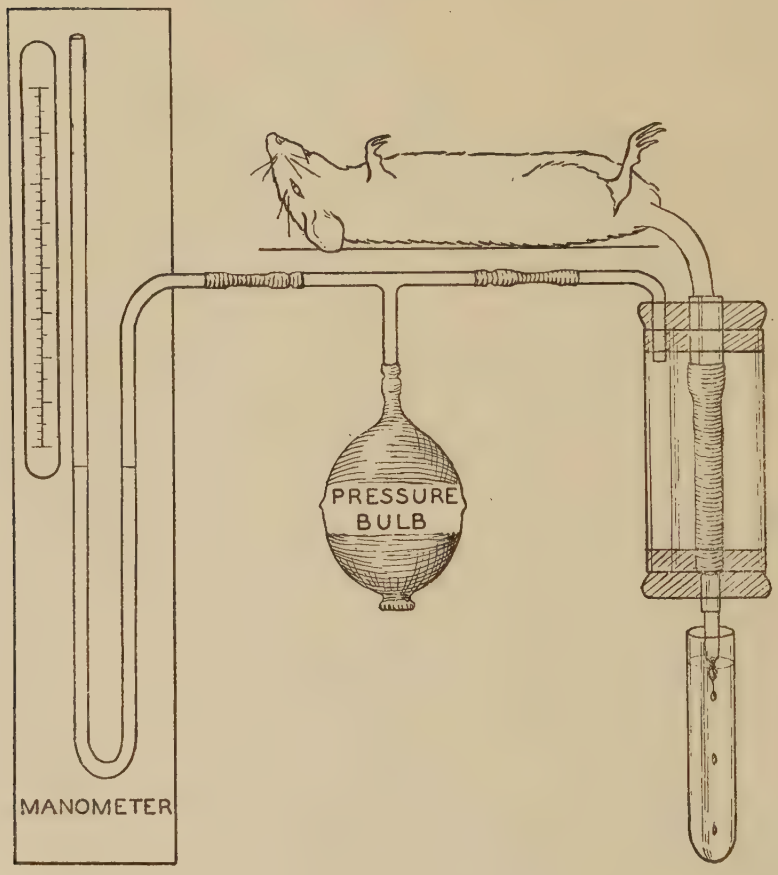
1 Griffith, J. Q., Jr., PROC. SOC. EXP. BIOL. AND MED., 1934-35, **32**, 394.

2 Bonsmann, M. R., *Arch. Exp. Path. u. Pharm.*, 1934, **176**, 460.

3 Hamilton, W. F., Woodbury, R. A., Harper, H. T., *J. A. M. A.*, 1936, **107**, 153.

4 Woodbury, R. H., and Hamilton, W. F., *Am. J. Physiol.*, 1937, **119**, 663.

FIG. 1
BLOOD PRESSURE CUFF AND MANOMETER
WITH RATS TAIL IN PLACE



As the pressure in the cuff is slowly released, the character of the stream of blood may be noted to change at 2 points. These points may be called "systolic" and "diastolic", although it is open to question whether this designation would be strictly correct. The "first point" is marked by the appearance of jets of blood which have the appearance of beads as they fall through the citrate solution. The flow of blood gradually grows larger as the cuff pressure is reduced. Suddenly there is a striking increase in the stream of blood. This is quite definite in most animals and is the "second point." The pressure in the cuff is raised again and the procedure repeated. After

the final reading, the end of the tail is ligated to prevent further bleeding.

Comparisons of blood pressure determinations by the indirect and direct methods were made, the latter being determined immediately after the former method.

Results. The results of determinations of blood pressures in 53 normal rats are shown in Table I. It was found that 87% of the "first point" pressures were between 101 and 130 mm mercury and 90.6% of the "second point" pressures were between 41 and 80 mm mercury. These pressures determined by the tail method vary less than those reported by Leiter[5] in a series of 131 rats in which the direct carotid artery method was used. He states: "Sixty-nine percent of the initial values fell between 101 and 140 mm Hg, 20% were above 140 mm Hg, and only 11% were below 101 mm Hg." A direct aortic blood pressure was obtained in 35 animals. The aortic pressures averaged approximately 10 mm Hg above our calculated mean of the "pulse pressure" (one-half of "pulse pressure" added to "second point" pressure). It is our impression that the so-called "second point" pressure appears to be less variable than the "first point" when compared with the blood pressure in the aorta obtained with mercury manometer and needle. The latter pressures agree with those reported by Leiter[5] and Durant,[6] who used the carotid artery. They also agree with the aortic pressures determined in 1,207 normal rats by Williams, Weigreia and Harrison,[7] who found that 94% of the pressures were between 90 and 140 mm Hg.

That the cuff readily detects changes in blood pressure produced by an injection of epinephrine is illustrated by the following experiments, 2 of which were performed. The control pressures were 160 "first point" and 90 "second point." At 3 and 5 minutes after the injection of epinephrine. the blood pressure had risen to 210 "first point" and 140 "second point." Thirteen minutes following the injection, the tail pressure had fallen to 180 "first point" and 120 "second point."

The indirect method described for taking blood pressures is similar to the one Chanuten and Ferris[8] attempted to use but discarded as unsatisfactory. The design of the cuff used in our experiments is probably the factor which has made the tail method satisfactory in our hands.

---

5 Leiter, L., *Arch. Int. Med.*, 1936, **57**, 729.

6 Durant, R. R., *Am. J. Physiol.*, 1927, **81**, 679.

7 Williams, J., Weigreia, R., and Harrison, T. R., in press.

8 Chanuten, A., and Ferris, E. B., *Arch. Int. Med.*, 1932, **49**, 767.

TABLE I.
Blood Pressures of Rats Determined by the Indirect Cuff Method and Direct Aortic Needle Puncture.
Normal Rats.

No. of Animals	Indirect Cuff Method						Mean Blood Pressure Calculated from "First Point" and "Second Point"	Direct Aortic Needle Method		
	"First Point"			"Second Point"						
	Avg	Min.	Max.	Avg	Min.	Max.		Avg	Min.	Max.
53	118	90	140	66	40	95	92	110	93	125

As previously mentioned, the points at which a change is noted in the character of the flow of blood from the cut end of the tail are designated as "first point" and "second point" for convenience. The so-called "second point" is usually extremely sharp and easily observed. It also appears to be less variable, when compared with the aortic blood pressure determined by the direct method of needle puncture. However, the "first point" pressure can be determined, we believe, particularly after some experience has been acquired with the same method. The "pulse pressures" we obtained in the present experiment are of the same order as those reported by Woodbury and Hamilton.[4] Both "first point" and "second point" pressures, particularly the "second point", are somewhat lower than the systolic and diastolic pressures given by Woodbury and Hamilton, but the fact that the tail vessels are quite distant from the heart may account for the lower values obtained. Direct pressures in the tail have not been recorded to our knowledge, so that the exact pressure in these vessels is not known.

Since the "second point" pressures we obtain can be determined easily, this figure alone, although perhaps somewhat low, would be useful in many types of experiments in which it is desirable to determine pressures at intervals for comparison. Landis believes that by heating the animal (*i. e.*, raising its body temperature) the tone of the vessels is lowered and, therefore, the blood pressure can be detected at a higher point. If this is the case, heating the animal, then, could supplement the method of blood pressure determinations reported here.

Summary. A method has been described for obtaining repeated determinations of blood pressure in rats. The blood pressures obtained with the indirect method agree with those obtained by the direct method of aortic cannulation.

10433 P

Effect of Castration on Body Weight and Length.*

H. S. Rubinstein, A. R. Abarbanel and A. A. Kurland.
(Introduced by J. C. Krantz, Jr.)
From the Research Laboratory, Sinai Hospital, Baltimore.

The recent availability of synthetic sex hormones for laboratory and clinical use has aroused new interest in their effect on somatic development. In an attempt to gather data concerning castration effects which could be used for comparison with stimulating or replacement effects of injected hormones, differences of opinion reported in the literature were found to be so striking[1] that it became necessary to establish our own standards for future comparison.

Accordingly, 32 male albino rats of Wistar stock were divided into 2 groups, one of which (16 animals) was castrated at 22 days of age while the other group of 16 served as unoperated controls. All animals were kept under similar conditions.[1] Weekly weights were determined for all animals while the body lengths were estimated (between the tip of the snout and anus) at the time of sacrifice on the 80th day of life. All data were statistically computed. In addition, theoretical body weight according to Formula No. 5 of Donaldson[2] was determined and this was compared with the observed terminal weight.

It was found that the initial body weight of 38.44±0.91 g for the castrates and 36.00±1.43 g for the normals were not significantly different from each other. The final mean body weight of the castrates was 192.0±3.85 g as compared to 217.88±3.40 g for the normal. This difference of 25.88±5.09 g in favor of the normal was probably significant. In addition, the final body length of 194.1±1.3 mm for the castrate as compared to that of the normal, 205.0±1.2 mm, showed a difference of 10.9±1.8 mm in favor of the normal. This, too, was a statistically significant difference.

The ideal final body weights calculated by formula disclosed a figure of 175.0±3.85 g for the castrates which was significantly less by 17.00±5.46 g from the observed weight (192.0±3.85 g). On the other hand, similar determinations carried out for the normal

* The authors acknowledge the coöperation of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study.

1 Rubinstein, H. S., Abarbanel, A. R., and Kurland, A. A., *Endocrinology*, in press.

2 Donaldson, H. H., *The Rat*, Wistar Institute of Anatomy and Biology, Philadelphia, 1924.

group disclosed a theoretical final body weight of 211.40±3.40 g. This figure was not significantly different from the observed weight for this group (217.88±3.40.).

These observations, namely a suppression of somatic growth as determined by body weight and body length, both of which were significantly inhibited, agree with the recent reports of Commins[3] and Lawless,[4] who also found an inhibition in body weight of castrated males. It may be added, however, that in contrast to the report of Lawless who, on the basis of comparison of body weight-body length (W/L^3) ratios concluded that weight and length were proportionately depressed, we feel that body length is affected more than body weight since at 80 days of age the castrate male had become relatively heavy for its smaller body length as determined by the formula of Donaldson.

10434

Absorption and Excretion of Sulfanilamidopyridine (2-Para-aminobenzenesulfonamidopyridine).*†

L. H. SCHMIDT AND HETTIE B. HUGHES. (Introduced by Glenn E. Cullen.)

From the Christ Hospital Research Institute and the Department of Biochemistry, College of Medicine, University of Cincinnati, Cincinnati, O.

This report concerns the absorption and excretion of sulfanilamidopyridine (2-para-aminobenzene sulfonamidopyridine) in 2 normal adult humans, following the oral administration of a single dose of the drug. Such data are of immediate interest since experimental studies,[1, 2] and preliminary clinical reports[3, 4, 5] indicate that sulfanilamidopyridine may be useful in the treatment of pneumococcus infections.

---

3 Commins, W. D., *J. Exp. Zool.*, 1932, **63**, 573.

4 Lawless, J. J., *Anat. Rec.*, 1936, **66**, 455.

* Sulfanilamidopyridine was supplied by Dr. David A. Bryce, The Calco Chemical Company, Bound Brook, New Jersey.

† The name of this drug has been officially changed to sulfapyridine.

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Hilles, C., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 73.

3 Telling, M., and Oliver, W. A., *Lancet*, 1938, **1**, 1391.

4 Evans, G. M., and Gaisford, W. F., *Lancet*, 1938, **2**, 14.

5 Flippin, H. F., and Pepper, D. S., *Am. J. Med. Sci.*, 1938, **196**, 509.

TABLE I.
Concentration of Sulfanilamidopyridine in Blood and Amount Excreted in Urine Following Ingestion of the Drug.

Hr after ingestion of drug	Normal Female—Wt 64 kilos Sulfanilamidopyridine						Normal Male—Wt 73 kilos Sulfanilamidopyridine					
	Conc. in Blood			Quantity in Urine			Conc. in Blood			Quantity in Urine		
	Free mg%	Conjugated mg%	Total mg%	Free mg	Conjugated mg	Total mg	Free mg%	Conjugated mg%	Total mg%	Free mg	Conjugated mg	Total mg
1	0.56	.04	0.60	—	—	trace	1.5	—	1.5	—	—	trace
2	1.00	.10	1.10	9	9	18	1.6	0.3	1.9	24	24	48
4	2.6	.30	2.90	36	56	92	2.1	0.6	2.7	44	82	126
8	2.0	.6	2.6	107	204	311	1.1	1.2	2.3	96	251	347
12	1.3	.8	2.1	88	214	302	0.6	0.9	1.5	44	166	210
24	—	—	trace	111	385	496	—	—	—	78	390	468
32				17	93	110						
				Total in 32 hr		1329				Total in 24 hr		1199

Each subject ingested 2 g of sulfanilamidopyridine suspended in 400 cc quantities of water. Blood samples were obtained 1, 2, 4, 8, 12, and 24 hours after ingestion of the drug; urine was collected quantitatively at the same intervals, and in one subject at the end of 32 hours. Both subjects took the drug one hour after breakfast and followed a normal routine of working, eating and sleeping.

Free and total sulfanilamidopyridine concentrations in blood and urine were determined colorimetrically according to Marshall's method[6] for estimation of sulfanilamide. Since sulfanilamide standards were used for comparison, the results were obtained in terms of sulfanilamide equivalents. These were converted to sulfanilamidopyridine concentrations by multiplying by the factors 1.25 in the free and 1.5 in the total sulfanilamidopyridine determinations.†

As the data in Table I show, the maximum concentrations of sulfanilamidopyridine in the blood, 2.7 and 2.9 mg %, were found 4 hours after the drug had been ingested. These concentrations fell rather slowly and at the end of 12 hours were 1.5 and 2.1 mg %. The relative proportions of free and conjugated sulfanilamidopyridine changed considerably during this interval. At 4 hours the conjugated drug was 10 and 22% of the total; at 12 hours this fraction amounted to 39 and 60%.

The data also show that urinary excretion accounts for between 60 and 65% of the sulfanilamidopyridine ingested. (The fate of the remaining 35 to 40% of the drug is still unknown; in view of its insolubility one may suggest that it has probably been lost in the feces.) Approximately 75% of the drug found in the urine was in the conjugated form, but the proportions of conjugated sulfanilamidopyridine were largest during the later periods of the experiment.

---

[6] Marshall, E. K., *J. Biol. Chem.*, 1937, **122**, 263.

† These conversion factors were derived experimentally. When sulfanilamidopyridine solutions were treated as in the determination of free sulfanilamide, the color produced amounted to 80.0% of that given by the same weight of sulfanilamide; the theoretical color equivalent is 69.4%. When sulfanilamidopyridine solutions were treated as in the determination of total sulfanilamide (*i. e.*, boiled in dilute acid for 90 minutes) the color produced amounted to 66.67% of that given by the same weight of sulfanilamide. These observations gave the conversion factors 1.25 ($^{100}/_{80.0}$) and 1.5 ($^{100}/_{66.7}$) for the free and total sulfanilamidopyridine determinations.

10435

Effect on the Electrocardiogram of Coumingine Hydrochloride, a New Alkaloid with Digitalis-like Action.

Stevens J. Martin and Bruce Cominole.

From the Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

Many substances have been found to have a digitalis-like action on the heart. However, until a few years ago, the only known alkaloid showing this effect was erythrophleine. Recently Chen[1] and his colleagues published observations on the potency of a series of natural cardiac *erythrophleum* alkaloids and one derivative. Based on the data in frogs and cats, coumingine hydrochloride, a salt of a new crystalline alkaloid of *Erythrophleum coumingo,*[2] proved to be the most potent of the whole group. Since the effects on the electrocardiogram of this alkaloid have not been studied, they have been made the subject of this investigation. A preliminary report of this study has appeared.[4]

The following experiments were performed: (A) Coumingine hydrochloride* was standardized by the Hatcher and Brody method[3] in 15 adult cats. (B) Electrocardiographic changes recorded during this procedure and also in 4 dogs were studied as well as those taken in another series of 23 dogs following daily intramuscular injections of various fractions of the cat unit per kilogram of body weight. In this latter group, injections were stopped after the onset of P-R prolongation and the daily EKG tracings continued until normal records were obtained. The electrocardiograms obtained in both the intravenous and intramuscular experiments were then compared with control records following injections of a standardized digitalis preparation in 6 cats and 7 dogs under similar conditions. (C) Toxic symptoms which appeared before the conclusion of experiments were noted throughout this study.

All animals showed normal EKG tracings before injections were started. Lead 2 was uniformly used although in many instances

1 Chen, K. K., Hargreaves, C. C., and Winchester, W. T., *J. Am. Ph. A.*, 1938, **27**, 9.

2 Dalma, G., *Boll. Soc. ital. biol. sper.*, 1936, **11**, 791.

3 Hatcher, R. A., and Brody, J. G., *Am. J. Pharm.*, 1910, **82**, 360.

4 Martin, S. J., and Cominole, B., *Am. J. Physiol.*, 1938, **123**, 141.

* Our sample was courteously supplied by Dr. K. K. Chen, the Lilly Research Laboratories, Indianapolis.

leads 1 to 4 were also employed. Dogs used in the intramuscular experiments were trained to lie quietly during EKG recording.

For the cat unit determination, a solution of coumingine hydrochloride crystals 1 :100,000 was prepared in warm saline and injected intravenously in cats at a rate of about 0.5 to 1.0 cc per minute. In dogs the strength of the solution for intravenous administration was 1:50,000 and the rate of injection 2 cc per minute. Electrocardiograms were taken at various intervals during a 90-minute period of injection, an average number of 15 readings being recorded for each animal. For daily intramuscular injections, a 1 :1000 solution was administered in order to keep the volume of injections small. Electrocardiograms were taken before and in many cases 1 to 4 hours after each daily injection until the end of the experiment. An average number of 20 tracings was recorded on each dog.

Results. A. *Determination of Cat Unit*—The cat unit of coumingine hydrochloride was found to be 0.159 mg per kg of body weight (range 0.102 to 0.184). This figure, which is in close agreement with that reported by Chen,[1] was used as a basis for the doses given in experiments on daily intramuscular injections. Emesis during intravenous injection of coumingine was noted in only 4 of 15 cats.

B. *Changes in the Electrocardiogram*—Electrocardiographic tracings taken every few minutes during the determination of the cat unit of coumingine hydrochloride closely simulated those obtained from digitalized preparations. In order, the following changes in lead 2 were noted in almost all animals studied (Fig. 1) : a widening and increase in height of T wave or commonly an absence or inversion of the wave, a progressive slowing of the S-A rhythm, bradycardia, P-R prolongation, intermittent absence of P waves, A-V dissociation, decrease in the height of the R complex with slurring and notching, showers of extraventricular systoles, ventricular tachycardia, and fatal ventricular fibrillation. Occasionally, there were noted extraäuricular systoles and rarely, a widening of QRS_2, and elevation of $S\text{-}T_2$. In the other leads, a slight occasional increase in Q_4 was found.

In the 15 cats and 4 dogs at the rate of intravenous injection of coumingine hydrochloride of about 0.007 to 0.02 mg per kg per minute, definite T-wave changes, P-R prolongation and A-V dissociation appeared on the average of 22, 44, and 53 minutes, respectively. Corresponding changes occurred more rapidly following digitalis injection.

The disturbances in the electrocardiogram of dogs receiving daily intramuscular injections of coumingine hydrochloride were similar

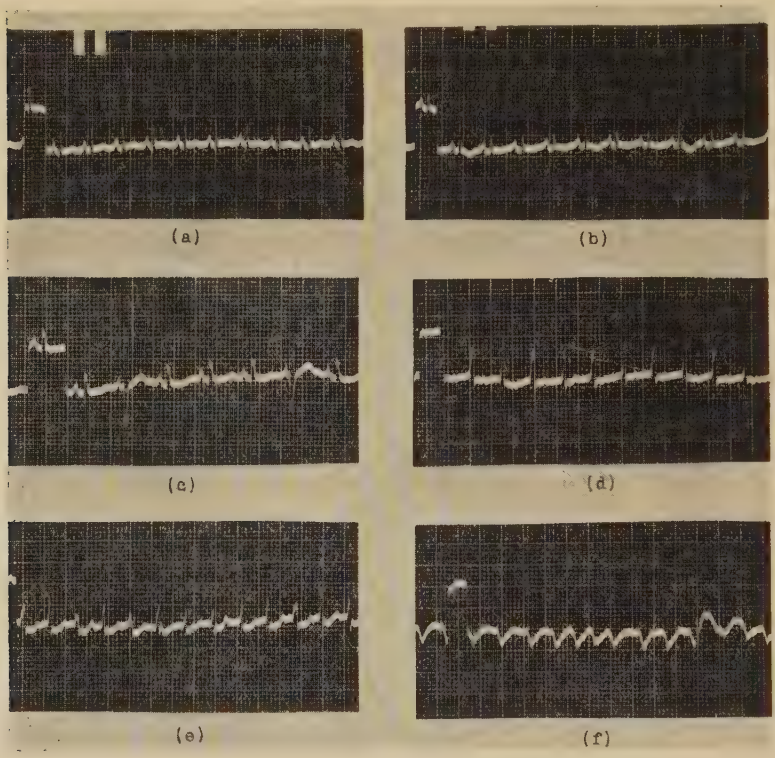


Fig. 1.

Successive electrocardiographic changes recorded on cat 14 during the determination of a unit of coumingine hydrochloride by the Hatcher and Brody method. Lead II: (a) control, (b) disappearance or inversion of the T waves (19 min.), (c) ventricular extrasystoles (25 min.), (d) P-R prolongation and splintering of R wave (46 min.), (e) complete A-V dissociation (50 min.), (f) ventricular fibrillation (67 min.).

to those noted after comparable doses of digitalis. However, there were differences in time of onset and disappearance of effects upon cessation of injection. On the whole, depression of the S-A mechanism and interference of nodal conduction was not as prompt with coumingine hydrochloride as with digitalis (Table I). With the large dose (1 cat unit per kg), P-R prolongation was generally noted within 2 hours when either drug was administered intramuscularly. With smaller doses, however, digitalis effects appeared predominantly earlier. This is true also for the appearance of widened and inverted T waves. After injections were stopped, the return of normal nodal conduction was not significantly faster in dogs given digitalis. The restoration of the T waves, however, was definitely more rapid in those receiving coumingine hydrochloride.

TABLE I.
Comparative Effects of Intramuscular Injections of Coumingine Hydrochloride and Digitalis on Electrocardiogram of the Dog.

Drug injected	No. of dogs used	Dose, cat unit per kg	No. daily injections Avg	Avg time before P-R prolongation, Days Hr	Avg days required for return to normal of EKG after cessation of injections		Remarks
					P-R prolongation	T wave changes	
Coumingine HCl	3	1.0	1	1-2	1.8	16	Total dose given in 2 equal injections 6-8 hr apart. Marked upper gastrointestinal symptoms after first administration.
Digitalis	3	1.0	1	1-2	2.0	22.5	One dog found dead third day after injection.
Coumingine HCl	6	0.3	5.2	5.2	5.1	13.3	Salivation and emesis after 2-5 daily injections. One dog found dead seventh day.
Digitalis	3	0.3	3.6	3.6	5.6	15.6	Nausea and emesis more marked in this group of dogs.
Coumingine HCl	3	0.2	9	9	7.7	14.7	Salivation and emesis only occasionally seen during end of injection period.
Coumingine HCl	3	0.1	16.7	21	4.0	15.6	Diarrhea in 2 dogs after eighth day.
Digitalis	2	0.1	10	11	3.5	21	Occasional emesis seen near end of injection period.

C. *Toxic Symptoms*—Muscular weakness of the leg probably due to pain resulted immediately after intramuscular injection of doses greater than 1 mg per kg. In 24 to 48 hours swelling of the thigh appeared with inflammation and subsequent serosanguinous discharge. Upon cessation of administration, all irritation subsided with no induration. None of the control dogs receiving digitalis intramuscularly in equivalent doses showed local reactions.

Evidence of general toxicity following daily intramuscular injections of coumingine hydrochloride consisted essentially of upper gastro-intestinal irritation manifested by salivation, anorexia, retching, and emesis. Other symptoms, less commonly noted, were transient tachypnoea 1 to 2 hours after injection, mild diarrhea and a variable loss in body weight. The onset of symptoms depended directly upon dosage. They were shown by all dogs receiving sufficient coumingine hydrochloride to induce definite P-R prolongation and were of milder character than those noted in control digitalized dogs.

The cardiac effects of erythrophleum alkaloids at present are merely of academic interest. Our data indicate that in addition to the pharmacological findings of Chen[1] coumingine hydrochloride can induce electrocardiographic changes similar to those of digitalis.

The similarity in these changes may possibly be accounted for by the presence of the sterol ring system in both compounds. However, it will require further chemical research to demonstrate the intimate structural relationship of coumingine hydrochloride to digitalis glucosides.

Conclusions. 1. The parenteral administration of coumingine hydrochloride, a new alkaloid, produced electrocardiographic disturbances in dogs and cats similar to those of digitalis. 2. The cat unit by the Hatcher and Brody method was found to be 0.159 mg per kg, confirming the result of Chen. 3. Daily intramuscular injections in dogs of variable sublethal doses of coumingine hydrochloride revealed that the onset of electrocardiographic disturbances was slower than after comparable doses of digitalis. The rate of disappearance of P.R prolongation after injections were discontinued, was essentially the same. However, the myocardial irritation of coumingine hydrochloride indicated by T-wave changes, was less persistent than after digitalis administration. 4. Toxic symptoms were essentially similar to those noted in digitalized controls but were milder in character. 5. Local inflammatory reaction following intramuscular injection of moderate or large doses of coumingine hydrochloride was marked.

10436 P

Effect of Luteinization on the Survival of Adrenalectomized Rats.

MARIE C. D'AMOUR AND FRED E. D'AMOUR.

From the Biologic Research Laboratory, University of Denver.

Several papers by Gaunt and his coworkers have recently appeared reporting upon the efficacy of progesterone in maintaining the life of adrenalectomized animals. We felt it would be interesting to determine whether one could induce a sufficient secretion of this hormone by the ovaries themselves to produce a similar effect.

Rats 35 days old at the time of adrenalectomy and weighing about 50 g were used. During the 10 days preceding adrenalectomy the ovaries were stimulated by injecting Gonadin* in doses of 2 r.u. or 6 r.u. daily per rat, for the first 5 days, and the same doses of Follutein* for the ensuing 5 days. The effect of Gonadin is chiefly upon follicular development while Follutein has mainly a luteinizing action. At the end of this preparatory period the ovaries of the animals on the larger dose weighed about 200 mg, those on the smaller dose, 20 mg. No further injections were given following adrenalectomy. The results are shown in Table I.

The results indicate that the progestin secreted by the corpora lutea of these animals significantly lengthened their time of survival. If the ovaries are removed at the time of adrenalectomy the survival time is no greater than that of the controls; the secretion of testosterone induced by similar stimulation is ineffective. Gaunt, Nel-

TABLE I.

No. of Rats	Treatment	% surviving	
		After 10 days	After 20 days
10 Females	None	0 (Avg, 5.6 days)	
10 "	2 r.u. Gonadin, 2 r.u. Follutein, each, daily, for 5 days	80 " 12.3 "	
5 "	As in previous group; ovaries removed at adrenalectomy	0 " 5.2 "	
10 "	6 r.u. Gonadin, 6 r.u. Follutein, each, daily, for 5 days	80	50
5 "	As in previous group, ovaries removed at adrenalectomy	0 " 5.2 "	
10 Males	As in fourth group	0 " 5.8 "	

* For the Gonadin and Follutein used, the authors are indebted to Dr. Wonder of the Cutter Laboratories and Dr. Morrell of E. R. Squibb and Sons.

son, and Loomis[1] state that 1-2 mg of crystalline progesterone daily are required to maintain the adrenalectomized rat. It is interesting, in view of the toxic effects of estrin in such animals, that the flourishing corpus luteum produces a sufficient amount of progestin to replace cortical hormone and also to overcome the effects of whatever estrogenic substance is being produced. The ovaries of the animals on the lower dose were apparently able to secrete progestin equivalent to 5% of their own weight daily. Further experiments are under way to determine, by means of continued injection, for how long a time the corpora are able to maintain this high level of progestin output.

10437 P

Sulphanilamide and Related Compounds in Experimental Tuberculosis.

P. H. GREEY, G. D. M. BODDINGTON AND M. H. LITTLE. (Introduced by W. L. Holman.)

From the Department of Pathology and Bacteriology, University of Toronto, and the Toronto General Hospital.

It was previously[1] shown that sulphanilamide—300 mg per animal per day—had an inhibitory effect upon the development of experimental tuberculosis in the guinea pig when the drug was administered before the injection of virulent human tubercle bacilli. These results were similar to those of Rich and Follis,[2] and Buttle and Parish.[3] However, we found no difference in the extent of macroscopic tuberculosis in treated and control animals when sulphanilamide was administered 17 and 24 days after the establishment of the tuberculous infection. But Ballon and Guernon[4] did find definite differences when the drug was given both 5 and 10 days after the infection. The latter 2 authors also found some evidence of bacteriostasis *in vitro*.

[1] Gaunt, Robert, Nelson, W. O., and Loomis, Eleanor, PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 319.

[1] Greey, P. H., Campbell, H. H., and Culley, A. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 22.

[2] Rich, A. R., and Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77.

[3] Buttle, G. A. H., and Parish, H. J., *Brit. M. J.*, 1938, **2**, 776.

[4] Ballon, H. C., and Guernon, A., *J. Thoracic Surg.*, 1938, **8**, 184 and 188.

Dietrich[5] has recently reported that Prontosil soluble does not modify the tuberculous process in guinea pigs even when given before the animals were infected. Similarly Kolmer, Raiziss and Rule[6] found that sulphanilamide and 6 derivative compounds given intramuscularly had no demonstrable beneficial effect in the treatment of experimental tuberculosis of guinea pigs. However, their method of administration of sulphanilamide and dosage were not entirely the same as those used in our experiments. Buttle and Parish,[8] using a bovine strain of tubercle bacilli, found that sulphanilamide had but little effect in guinea pigs, and none in rabbits.

The present report deals with a comparative study of the effect of sulphanilamide and related substances upon the tuberculous process in the guinea pig and rabbit. The following substances were studied and their effect compared with the result obtained with sulphanilamide: Prontosil soluble, Prontosil base, the dimethyl derivative of disulphanilamide (Uleron), diacetyl diamino diphenyl sulphone and p-benzylamino benzenesulphonamide (Septazine).*

(a) *In Guinea Pigs.* The total daily dose of the drugs was divided into 4 equal doses and administered at 9 a.m., 1 p.m., 5 p.m., and 10 p.m. *per os* by means of a nasal speculum. The drugs were administered in the following amounts per guinea pig per day: sulphanilamide, diacetyl sulphone, Uleron and Septazine 300 mg each, Prontosil base 50 mg, Prontosil solution 2 cc of a 2½% solution. Eight guinea pigs were used for each compound while 20 untreated animals served as controls. The various compounds were administered for 3 days, then all the animals were infected by the subcutaneous injection of 0.2 cc of a suspension of virulent human tubercle bacilli containing 2-6 microörganisms per oil immersion field. Only multicolored guinea pigs weighing about 350 g were used. At death the treated animals were necropsied and the macroscopic and microscopic findings were compared with those of the sulphanilamide-treated and control animals that had been infected for a similar period of time.

Again it was found that in the animals treated with sulphanilamide the lesions in the spleens and livers were small and few in number in contrast to the many prominent tubercles in the control

5 Dietrich, H. F., *Am. Rev. Tuberc.*, 1938, **38**, 388.

6 Kolmer, J. A., Raiziss, G. W., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 581.

* The following compounds were generously supplied by: Winthrop Chemical Co., Prontosil base, sulphanilamide, Uleron and diacetyl sulphone; Poulenc Frères, Ltd., Septazine and diacetyl sulphone; E. B. Shuttleworth Chemical Co., sulphanilamide.

animals. The lesions in the animals treated with the other compounds, however, were equal to and in some instances exceeded in number and size those in the controls.

All the compounds proved to be toxic in the doses used, in that half of the treated animals died before the end of the fourth week and on several occasions it was found necessary to decrease the daily dose of the compounds for 2 days in order to overcome toxic symptoms.

(b) *In Rabbits.* In this experiment each of the aforementioned compounds were administered to 2 rabbits 4 times daily at the same hours as the guinea pigs were treated. Six rabbits served as controls. The compounds were administered for 3 days and then all 18 rabbits were infected by an intravenous injection of 1 cc of a light suspension of virulent bovine tubercle bacilli. The rabbits selected for use weighed about 1.2 kilos and the compounds were administered in the following total daily dose per animal: sulphanilamide, diacetyl sulphone, Uleron and Septazine 1 g, Prontosil base 200 mg and Prontosil solution 8 cc of a 2½% solution.

The treated and untreated animals died during the third to fourth weeks after the injection of bovine tubercle bacilli. At necropsy all the rabbits showed widespread tuberculous infection of the lungs and no difference could be noted between the lesions of the treated animals and of the controls. Several microscopic sections were prepared from the kidneys of both the treated and untreated animals. Examination failed to reveal any difference in the renal lesions in the treated animals from those occurring in the controls.

Conclusions. Under the conditions of the experiment sulphanilamide was again found to inhibit the tuberculous process in guinea pigs infected with human tubercle bacilli. The large doses necessary to obtain such results preclude clinical application. In guinea pigs similarly infected but treated with Prontosil soluble, Prontosil base, the dimethyl derivative of disulphanilamide (Uleron), diacetyl diamino diphenyl sulphone and benzylamino benzene sulphonamide (Septazine) the tuberculous process was not inhibited. The above compounds, including sulphanilamide, exerted no influence upon the course of the tuberculous infection induced in rabbits by the intravenous injection of bovine tubercle bacilli. The renal lesions in the treated and control rabbits showed no differences.

10438 P

Effect of Testosterone Propionate on Development and Growth of Mammary Carcinoma in Female Mice.*

IRA T. NATHANSON AND HOWARD B. ANDERVONT. (Introduced by J. C. Aub.)

From the Collis P. Huntington Memorial Hospital, Harvard University, and the Office of Cancer Investigations, U. S. Public Health Service.

Estrogen, when administered to male mice of certain strains, will result in the appearance of spontaneous mammary carcinoma.[1, 2, 3] The females of these strains ordinarily have a high incidence of spontaneous mammary carcinomas, which do not normally occur in the males. It has further been demonstrated that the incidence can be increased and the time of appearance of the mammary carcinoma hastened by the administration of estrogen to the females of these same strains.[4] Recently, Lacassagne[5] tested the effect of testosterone upon development of mammary carcinoma in females of a high incidence strain and noted no alteration from the controls. The dosages used, however, were small.

It is the purpose of this communication to report the results of long term injections of testosterone propionate* upon subsequent development and growth of spontaneous mammary tumors in female mice with an extremely high incidence (95%).[6]

Forty female mice of the C_3H strain, born at approximately the same time, were kept under similar experimental conditions. They were then bred at maturity and all except 2 animals had their litters within several weeks of each other. The remaining 2 animals had litters one month after the first of the entire group. The litters were killed within 24 hours after birth and the animals were then divided into 2 groups of 20. For every animal in one group, there was a sister litter mate in the other. At the age of 4½ months, injections

1 Lacassagne, A., *Am. J. Cancer*, 1936, **27**, 217.

2 Gardner, W. U., Smith, S. M., Strong, L. C., and Allen, E., *J. A. M. A.*, 1936, **107**, 656.

3 Burrows, H., *Brit. J. Surg.*, 1935, **23**, 191.

4 Suntzeff, V., Burns, E. L., Moskop, M., and Loeb, L., *Am. J. Cancer*, 1936, **27**, 229.

5 Lacassagne, A., *Compt. rend. Soc. de Biol.*, 1937, **126**, 385.

* We are indebted to Doctors Gregory Stragnell and Max Gilbert of the Schering Corporation for the generous supply of testosterone propionate (Oreton) which was used in this experiment.

6 Andervont, H. B., and McEleney, W. J., *Public Health Reports*, 1937, **52**, 772.

of testosterone propionate in sesame oil were given to one group of 20, dosage 0.5 mg in 0.05 cc of oil 3 times weekly for a period of 4 months. The second group served as controls, and were given 0.05 cc of sesame oil, without the hormone, 3 times a week.

Only the final results will be given as this experiment and others will be reported in detail at a later date. Of the 20 treated animals, six (30%) only developed spontaneous mammary carcinoma. All of these arose within 4 months after injections were commenced. These were single tumors and all animals died by the 11th month of life without the development of a second tumor. Four mice of the group died in the 12th month of life without any evidence of tumor at autopsy. Ten are still living and healthy without tumors at 16 months of age. The testosterone had no effect upon the growth rate of the six tumors which arose during the period of injections, when compared with the control tumors.

In the control group, all of the animals (100%) developed one or more tumors by the 11th month of life. The distribution of tumors was as follows: 8 mice had one tumor; 7 had 2 tumors; 4 had 3 tumors; and 1 had 5 tumors. All animals died from tumors by the 14th month of life.

The effect of large doses of testosterone propionate on the tumor *per se* was then tried on a series of 40 female mice in which tumors had already developed. These tumors ranged in size from 3 mm to 18 mm and were divided into 2 groups as nearly identical as possible. Group No. 1 was given 1.0 to 2.5 mg of testosterone propionate in 0.05 to 0.1 cc of sesame oil daily for 4 weeks. Group No. 2 (Control Group) was treated with 0.05 to 0.1 cc of sesame oil daily. Measurements were made in 3 diameters every third day until death. No difference in growth rate could be detected between the treated and untreated animals.

Testosterone *per se* has no effect on the growth of spontaneous mammary carcinoma in the female of the C_3H strain mice. Testosterone, when administered at an early age (4½ months or younger) to animals that have had one litter, will prevent the development of tumor provided there were none present when the treatment became effective. It is assumed that those tumors which developed were microscopic when treatment was commenced and were not influenced in their growth rate.[7, 8]

[7] Shaw, D. T., and Nathanson, I. T., unpublished data.

[8] Nathanson, I. T., and Shaw, D. T., unpublished data.

10439

Effect of a Pellagra-Producing Diet on Herbivora.*

P. B. Pearson, H. Schmidt and A. K. Mackey.

From the Department of Animal Husbandry and Division of Veterinary Science, Agricultural Experiment Station, Agricultural and Mechanical College of Texas, College Station.

Investigations have shown that nicotinic acid is effective in the prevention and cure of human pellagra[1] and black tongue in dogs.[2] Recently Chick, *et al.*,[3] and Hughes[4] have shown that nicotinic acid is a dietary essential for pigs. Aside from vitamin B_1, information is lacking on the requirements of Herbivora for various members of the vitamin B complex. Bechdel, *et al.*,[5] obtained normal growth of calves on a ration that provided insufficient vitamin B_1 to support growth in rats. Bacteria present in the rumen of calves fed a B_1 low ration were found to be highly potent in promoting growth of rats maintained on a B_1-deficient ration.[6]

The present paper reports the results of investigations on the rôle of nicotinic acid in the nutrition of Herbivora. Sheep were used as the experimental animal in this investigation.

Six weanling lambs between 2 and 3 months of age and weighing between 13.6 and 18.8 kilos were placed on a ration consisting of regenerated cellulose 20, yellow corn 60, peas *(Vigna sinensis)* 10, unpurified casein 9, dicalcium phosphate 1, and sodium chloride *ad libitum*. Vitamins A and D in addition to that furnished through natural sources were supplied by oleum percomorphum. Since Birch, *et al.*,[7] had produced pellagra-like symptoms in pigs, using unpurified casein, we were prompted to use a similar source of protein in our ration for lambs.

---

* Published with the approval of the Director of the Texas Agricultural Experiment Station as technical contribution No. 506.

1 Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 405; Smith, T. D., Ruffin, J. M., and Smith, S. G., *J. Am. Med. Assn.*, 1937, **109**, 2054.

2 Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Biol. Chem.*, 1938, **123**, 137.

3 Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., *Biochem. J.*, 1938, **32**, 10.

4 Hughes, E. H., *Hilgardia*, 1938, **11**, 595.

5 Bechdel, S. I., Eckles, C. H., and Palmer, L. S., *J. Dairy Sc.*, 1926, **9**, 409.

6 Bechdel, S. I., Honeywell, H. E., Dutcher, R. A., and Knutsen, M. H., *J. Biol. Chem.*, 1928, **80**, 231.

7 Birch, R. W., Chick, H., and Martin, C. J., *Biochem. J.*, 1937, **31**, 2065.

All of the lambs made good growth for the first 4 or 5 weeks. Following this there was a rapid decline in weight, with a concomitant decrease in the amount of feed consumed. Some of the animals lost as much at 4.5 kilos, or 24% of their weight. At this point 5 mg of nicotinic acid† per kilo of body weight was administered daily *per os* to Nos. 270 and 300. While there is no information to indicate that vitamin B_1 is necessary for this species, conclusive evidence that it is not, is lacking. In order to eliminate any possibility of B_1 being a limiting factor in the ration, a solution of thiamin was administered *per os* to Nos. 253 and 297 at the rate of 50 micrograms daily per kilo of body weight. Subsequently both the treated and untreated animals recovered with equal rapidity and made satisfactory growth thereafter. Whether this temporary failure reflects a period during which the animal organism was making an adjustment to the deficiency, perhaps through an increase in microörganisms in the rumen that might synthesize nicotinic acid or to some other factor, cannot be answered at present.

The lambs were continued on this ration for a total of 130 days. The lambs that received nicotinic acid or vitamin B_1 made no better gains than those not given a supplement. Therefore, the addenda were discontinued after 44 days.

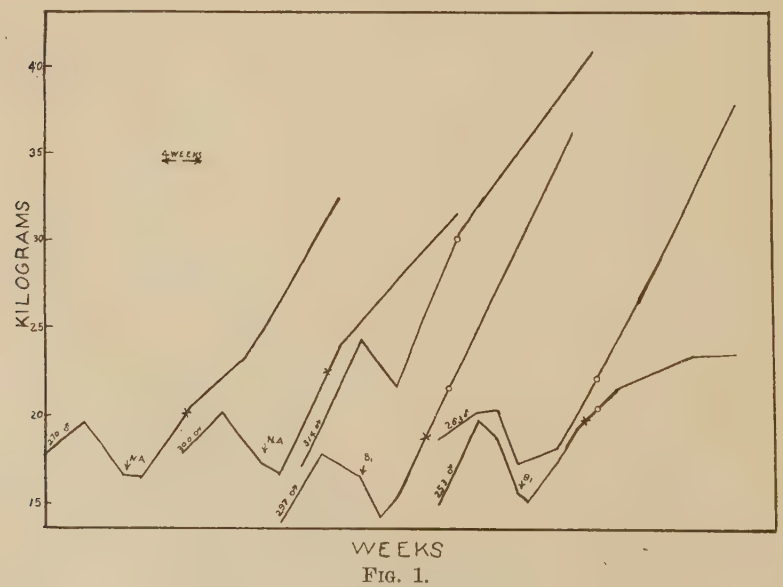


FIG. 1.

Growth curve of lambs on pellagra-producing ration. Nicotinic acid and B_1 discontinued at x. Circle indicates point at which animals were changed to a ration containing purified casein.

† Supplied through the courtesy of Merck & Company.

At the end of the 130-day period the 4 lambs that had not received nicotinic acid were changed to a ration in which the unpurified casein was replaced by "Labco Vitamin-free Casein." These lambs were fed this ration for 90 days, while Nos. 270 and 300 were continued on the ration containing unpurified casein. During this period all of the lambs except No. 253 made excellent gains of between 114 and 164 g per day. Since No. 253 made no improvement after being placed on a standard fattening ration at the conclusion of the experiment it is believed that his failure to make as good growth as the others was due to causes other than the nature of the ration fed. There was no significant difference in the performance and rate of growth between the lambs fed the ration containing the unpurified casein and those fed the ration containing the purified casein, which is a typical pellagra-producing ration. The gains made by the lambs in both groups were comparable with the gains of lambs of similar weight on a well-balanced fattening ration.

Summary. Lambs developed normally on a ration that causes cessation of growth and produces pellagra-like symptoms in pigs and black tongue in dogs. From this it appears that nicotinic acid is either not a dietary essential for this species or that the requirements are much lower than for the pig, dog, or human.

10440

A New Fibrinogen Preparation.*

John H. Ferguson and B. Nims Erickson.

From the Departments of Pharmacology and of Pediatrics, University of Michigan, Ann Arbor.

In a recent presentation of a series of standardized procedures for the *in vitro* study of coagulation reactions,[1] it was noted that the essential thrombin components (prothrombin, calcium, cephalin) could all be obtained in stable form and readily converted into appropriate solutions. The fibrinogen, on the other hand, was always freshly prepared, consuming much valuable time and risking a poor preparation from denaturation and other causes. In searching for a method of obtaining a stable "stock" preparation of this labile

* One of us (B. N. E.) begs to acknowledge the receipt of a Sigma Xi research grant in aid of the accompanying investigation.

1 Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

protein, it was recalled that most modern serological laboratories are equipped with a high-vacuum, low-temperature desiccating apparatus for the dehydration of sera and similar preparations. Through the courtesy of Dr. M. H. Soule of the Bacteriology Department of the University of Michigan, we were enabled to use such an apparatus in the investigation of the present problem.

Fibrinogen was "salted-out" from fresh citrated dog plasma by the usual methods. The final deposit from 50-60 cc of the original plasma was firmly packed at the bottom and adjacent side in *each* of a number of 50 cc centrifuge tubes, the angle centrifuge being utilized because of the advantage of the large sloping surface. With complete removal of the gross supernatant fluid by suction and drainage, the water content of the deposited protein was relatively small. Eight tubes could be handled at a time, their contents representing the fibrinogen from some 500 cc of citrated plasma or, approximately, a liter of blood. The tilted tubes were immersed in a mixture of "dry ice" and "methyl cellosolve" and the contents rapidly solidified without noticeable interference by the adherent precipitating salt. By distillation at high vacuum (20-40μ) into an intervening condenser tube surrounded by the freezing mixture (in a vacuum flask), the fibrinogen was reduced to a dry flaky powder in a few hours (overnight, by preference). On redissolving in distilled water or 0.9% NaCl, with enough 0.5% $NaHCO_3$ to render alkaline to phenol red, a gradual resolution of the bulk of the protein occurred. Filtration yielded a clear opalescent solution indistinguishable in properties from the original *fibrinogen* solution. It clotted excellently on the addition of an appropriate coagulant. Traces of *prothrombin* from the original preparation were also apt to be preserved as shown by slow coagulation with calcium and cephalin alone.

Among several modifications of the method which were tried the following may be mentioned:

1. Berkefeld-filtered citrated dog plasma was precipitated with $(NH_4)_2SO_4$ (one-quarter saturation) and the redissolved fibrinogen reprecipitated 3 times. The desiccated preparation was satisfactory for routine clotting tests where the trace of prothrombin present could be ignored. After several months, good resolution is still being obtained although there is a gradual increase in denaturation. From the amount of dilute alkali needed to restore the pH, it is surmised that there is sufficient acidity in the ammonium salt to favor such denaturation.

Analyses for total N and N.P.N. by the Van Slyke technic indicated that some 90% of the nitrogen present belonged to the precipi-

tating salt. Besides being too high a concentration of N.P.N. to permit accurate protein estimation by N-analyses, this amount of ammonium salt has a certain measure of inhibitory action on the clotting phenomena.

We have routinely used *Berkefeld-filtered* plasma[1] in order to remove all possible traces of platelet and similar material. Analytical comparisons with twice-centrifuged plasma showed a minor reduction (5-6%) in total N and no loss of phospholipids. While the ultrafiltration may be dispensed with for many purposes, the facts that good fibrinogen and prothrombin preparations may be made from the filtrate accord with these chemical analyses in favoring the view that the plasma is not greatly disturbed by such filtration when properly conducted.

2. By the use of *sodium* salts and greater care to keep the fibrinogen at ice-cold temperatures during the course of preparation (Florkin[2] recommends the cold room at 1-2°C) it should be possible to control denaturation and obtain a protein free from N.P.N. The fibrinogen prepared with sodium salts is in our experience always very gelatinous and tenacious and much less easily recovered (by centrifugalization) and redissolved than that prepared with $(NH_4)_2SO_4$.

We, therefore, recommend a "combined method" in which 3 ammonium sulphate precipitations are followed by one with NaCl. If desired, the final precipitate, after centrifuging, is resuspended and washed once or twice in half-saturated NaCl solution. The dehydrated preparation from this material appears to keep better, to redissolve more completely (although equally slowly), and to yield solutions with N.P.N. values down to 5-10%, or lower, thus admitting of accurate protein analysis.

3. A well-washed, N-free Alumina *Gel* (ortho Al $(OH)_3$, β-form[3]) was found to adsorb all the protein from redissolved weak fibrinogen solutions. It was, therefore, found practicable to prepare *prothrombin-free* fibrinogen only by adsorbing the original citrated plasma[1] and subsequently salting-out the fibrinogen. The desiccated preparation gave (filtered) solutions entirely free from all traces of prothrombin. The material subjected to such additional handling did not keep as well as the other preparations. Nevertheless, the undenatured (soluble) portion could be used for clotting tests even after several months.

---

2 Florkin, M., *J. Biol. Chem.*, 1930, **87**, 629.

3 Method cited in C. Oppenheimer's *Die Fermente und ihre Wirkungen*, 1929, III, p. 480.

Summary. A method is described for dehydration *(in vacuo)* of salted-out fibrinogen for "stock" usage of the protein in coagulation studies. Modifications of the method of preparation are considered with a view to (a) lessening denaturation, (b) making the preparation available for biochemical analysis of its protein content, and (c) preparing it free from all traces of prothrombin.

10441 P

Urolithiasis and Renal Pathology After Oral Administration of 2(sulfanilylamino)pyridine (Sulfapyridine).

WILLIAM ANTOPOL AND H. ROBINSON.

From the Laboratories of the Newark Beth Israel Hospital, Newark, New Jersey, and the Merck Institute of Therapeutic Research, Rahway, N. J.

In the course of an investigation of the pharmacology and toxicology of 2(sulfanilylamino)pyridine, which will be reported elsewhere,* we have observed the formation of uroliths in the urinary tract of rats, rabbits, and monkeys fed with this drug, which were found to consist of its acetyl derivative having the formula $CH_3CONHC_6H_4SO_2NHC_5H_4N$. The appearance of sulfanilamide crystals in the urine of patients has been previously reported by Stewart, Rourke and Allen.[1]†

While concretions have been observed after the administration of a single large dose, the results were more striking after repeated feeding on successive days. The occurrence of urolith formation following the repeated daily administration of 2(sulfanilylamino)-pyridine varied greatly in the different species and to some extent even in the same species. Thus uroliths were observed after feeding daily doses of 0.25 g per kilo to monkeys, 10 to 15 g per kilo to rab-

* By H. Mollitor and H. Robinson.

[1] Stewart, J. D., Rourke, G. M., and Allen, J. C., *J. Am. Med. Assn.*, 1938, **110**, 1885.

† Lawrence mentions a human case of right lower quadrant pain and hematuria due to stone formation after sulfapyridine therapy.[2] Oakley in a short note mentions the presence of prontosil in the bladder and the straight and collecting renal tubules of mice fed with enormous doses of this drug.[3]

[2] Lawrence, E. A., *International Review of Recent Advances in Medicine*, 1939, **3**, 48.

[3] Oakley, C. L., *Biochem. J.*, 1937, **31**, 729.

bits and 5 g per kilo to rats for 10 consecutive days, whereas the phenomenon was not observed in mice or dogs even after the feeding of such excess doses as 20 g per kilo. In a series of 25 monkeys fed doses of 0.25 to 14 g per kilo only one animal (receiving 10 g per kilo) failed to produce uroliths. Individual variation was found to be greater in a series of 30 rabbits and 120 rats, particularly with smaller doses (2 to 5 g per kilo) but the results were quite uniform when large doses (10 to 20 g per kilo) were employed. With small doses, urolith formation was more frequently observed on the right side, while the left ureter remained free of involvement. In contrast to the above results, daily feedings of sulfanilamide over prolonged periods of time produced a similar phenomenon only in 2 rats out of a series of over 300.

A few hours after the oral administration of a large dose of sulfapyridine, the urine in susceptible species is seen to contain needle-like crystals of the substance. This has already been found in rabbits by Stokinger.[4] If the animal is sacrificed 24 hours later, aggregates of these crystals are observed, especially in the ureter, most frequently at the level of the bony pelvic brim. At times a calculus may be found in the ureter, where it enters the bladder wall, or in the bladder proper. The proximal ureter and renal pelvis is at first somewhat dilated and thinned out. Later these structures become indurated and hemorrhagic. Bloody urine and blood clots are found within the dilated channels. In some instances the initial obstruction occurs at the uretero-pelvic junction. In these cases an amorphous mass composed of compact crystals completely fills the pelvis and extends into the medulla of the kidney. The kidney proper becomes edematous and increases in size, varying from slightly larger than normal to twice the normal size.

The histology of the kidney varies with the degree of urinary stasis. The early picture is that of a calculus ureteritis and pyelitis; and with involvement of the kidney substance, a pyelonephritis ensues. In the more pronounced cases, there is marked retention of nitrogenous products in the blood.

There is in the experimental data a suggestion that the crystallized compound, when not excessive can either be redissolved or washed out. In a series of 5 monkeys, all of which received 4 g per kilo of sulfapyridine for 10 days, 4 of the animals which were sacrificed at the last day of feeding showed marked formation of uroliths while the fifth, sacrificed 79 days after discontinuation of the drug, exhibited a definite thickening and dilatation of the ureters and

[4] Stokinger, H. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 61.

pelves, but no formation of concrement. This question is receiving further attention.

The uroliths consisting of the acetyl derivative of sulfapyridine permit penetration by X-rays. However, it has been observed that calcium can be deposited about these concrements which act as a nucleus in which case the shell may become X-ray opaque.

10442 P

Production of Mammary Carcinomas in Male Mice With a Single Implantation of Oestrone.

GRAY H. TWOMBLY. (Introduced by J. Ewing.)

From the Memorial Hospital, New York City.

Lacassagne[1] produced tumors in male mice of the R III strain, in which 70% of the females develop spontaneous mammary carcinomas, by the weekly injection of 300 I.U. of oestrone benzoate in oil. The tumors were found to develop sooner in males so treated than they occur spontaneously in females of the same strain. These findings have been repeated by Burrows,[2] by Bonser,[3] by Cramer and Horning,[4] and by Gardner, Smith, Allen and Strong.[5] Lacassagne[6] used oestrone, oestradiol, equiline, and equilinine, all hormones being injected weekly in oily solution. Burrows painted his animals twice weekly with oestrone, .01% in benzene; Bonser used oestrone benzoate in olive oil as did Gardner, *et al.*, while Cramer and Horning used oestrone in chloroform applied to the skin twice weekly.

Recently Deanesly and Parkes[7] have shown that prolonged effects may be obtained by the implantation of crystals of oestrone under the skin. A capon so treated with a 3 mg crystal of oestrone maintained the hen coloration of the breast feathers for over 3 months. It seemed that this method might be made use of to repeat the experiments of Lacassagne while decreasing the labor of weekly injec-

1 Lacassagne, A., *Compt. rend. Acad. de sc.*, 1932, **195**, 630.

2 Burrows, H., *Am. J. Cancer*, 1935, **24**, 613.

3 Bonser, G. M., *J. Path. and Bact.*, 1935, **41**, 217; 1936, **42**, 169.

4 Cramer, W., and Horning, E. S., *Lancet*, 1936, **1**, 247.

5 Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C., *Arch. Path.*, 1936, **21**, 265.

6 Lacassagne, A., *Bull. de l'assoc. fr. p. l'etude du cancer*, 1938, **27**, 1.

7 Deanesly, R., and Parkes, A. S., *Proc. Roy. Soc., B*, 1937, **124**, 279.

tions and removing any confusing results due to the application of such large quantities of solvent.

In order to determine whether prolonged action of oestrone could be obtained in mice by the implantation of oestrone crystals 7 stock females were selected and vaginal smears taken for a week to show that they were all ovulating normally. At the end of this time crystals of oestrone (obtained from the Schering Corporation through the kindness of Dr. Schwenk) weighing 0.68 mg to 1.06 mg recrystallized from ethyl alcohol were introduced beneath the skin of the abdomen of each animal with a trocar. Each animal received one crystal. Daily smears were taken and showed all the animals to be in constant oestrus until the day of death. Four animals died of pyometra at the end of 6 weeks. One was accidentally killed at the end of 3 months. One died with pyometra after 5 months and the last at 8½ months. The latter showed uterine horns so distended with fluid as to make the animal appear almost circular in outline.

Having demonstrated that single crystals of oestrone would produce constant estrus in female animals for at least 8 months, young male mice of the R III strain in which 70% of the females develop mammary tumors were selected at approximately 10 days of age and injected with crystals of oestrone beneath the skin in the pectoral region. A fine spinal puncture needle was found to make a very satisfactory trocar for this procedure. Twenty-one animals were so treated. The amounts of oestrone used in each animal varied from .06 mg (600 I.U.) to .2 mg (2000 I.U.). Sixteen of the animals died before 100 days, all showing signs of urinary obstruction (distended bladder, hydronephrosis, hydroureter) from prostatic enlargement and keratinization. Two animals are living to date (5 months) with no signs of oestrinism. In these animals the minute crystals of oestrin probably came out unnoticed with the needle. Of the remaining 3, one died at 6½ months without tumor and 2 developed large mammary carcinomas.

The first of these occurred in a male mouse injected with 0.1 mg of oestrone 4 months and 26 days before the appearance of the tumor in the right inguinal region. The mouse was killed 3 weeks after the tumor was first seen, at which time 2 smaller tumors were found in the axillary region. The tumor was transplanted to 6 male mice of the same strain and grew in all. Gross preparations of the breast tissue showed tremendous development (both duct growth and acinar proliferation) of the non-cancerous portions of the breast. The bladder was filled with phosphate crystals and there was bilateral hydronephrosis. The seminal vesicles were greatly enlarged and

weighed 404 mg. The pituitary weighed 2.3 mg, about twice the normal weight in this strain. The adrenals showed the brown degeneration described by Cramer[8] in animals treated with large amounts of oestrin.

The second tumor occurred in a mouse injected 5 months before with a single crystal of oestrone. This animal also showed a distended bladder and bilateral hydronephrosis but in contrast to the first animal the seminal vesicles were atrophied. There was a very marked enlargement of the pituitary to at least 5 times normal size. Histological sections showed this to be more a diffuse hypertrophy rather than an adenoma, all 3 types of cells lying scattered throughout the gland. The adrenal showed a less degree of brown degeneration than occurred in the first mouse. The breast tumor grew progressively in one of 2 animals into which it was transplanted, confirming the histological diagnosis of adenocarcinoma.

Fourteen virgin littermate females have shown as yet no spontaneous mammary tumors at 3 to 7 months of age. The treatment received by the 2 males developing tumors not only induced the formation of carcinomas but produced them sooner than any such tumors occurred spontaneously in their sisters. Mammary carcinoma does not occur spontaneously in male mice.

Since this article was submitted for publication, 13 additional male mice similarly treated have developed mammary tumors.

10443 P

Possible Effects of Vitamin K on Prothrombin and Clotting Time in Newly-born Infants.

WILLIAM W. WADDELL, JR., DUPONT GUERRY, III, WILLIAM E. BRAY AND ORVILLE R. KELLEY. (Introduced by J. R. Cash.)

From the Department of Pediatrics and the Department of Clinical Laboratories, University of Virginia Medical School.

Three deaths from hemorrhagic disease of the newly-born occurring recently on the pediatric service of the University of Virginia Hospital prompted studies to determine the normal prothrombin level in a group of newly-borns with the hope that some agent could be found which might materially effect prothrombin levels. Very early in this investigation 2 cases with abnormally high prothrombin

[8] Cramer, W., and Horning, E. S., *J. Path. and Bact.*, 1937, **44**, 633.

time and clotting time were observed. Because of the striking results which followed the administration of Vitamin K in these 2 instances, it seems wise to report our findings, the completed study to be reported at a later date. The 2 cases are recorded only as a preliminary report of work now in progress concerning prothrombin levels in the newly-born and the possible relationship of such levels to hemorrhagic disease of the newly-born.

Case I. Baby D. A female infant weighing at birth 3345 g was born 2-12-39 in the University of Virginia Hospital. On physical examination the infant appeared normal in every respect. On the third day after birth at 3:30 p.m. blood was removed from the longitudinal sinus. The prothrombin time was found to be 6 minutes. Blood from a puncture wound of the heel showed a coagulation time of 9 minutes. Slow oozing from the puncture wound of the heel occurred over a period of 12 hours. At 10:45 the following morning fontanel puncture was again done and the prothrombin time of the blood was found to be 7 minutes and the clotting time 11 minutes. Two cc of a concentrate rich in so-called Vitamin K was administered by mouth. Two hours later the prothrombin time had dropped from 6 minutes to 55 seconds and the clotting time had dropped from 11 minutes to 4 minutes. Oozing from puncture wound of heel ceased promptly after administration of Vitamin K. Throughout hospital stay prothrombin time and clotting time remained consistently low and on the seventh day prothrombin time was 23 seconds and clotting time was 3 minutes.

Case II. Baby girl T. Born in the University of Virginia Hospital 2-12-39 of a luetic mother who had received suitable treatment. At the time of the baby's birth, the mother's Wassermann test was negative. On physical examination the infant appeared normal in every respect and the weight was 2770 g. On the afternoon of February 15, the fourth day of life, the prothrombin time was found to be 3½ minutes and the coagulation time 11½ minutes. Two cc of a concentrate rich in so-called Vitamin K was administered by mouth. Two hours later the prothrombin time had dropped from 3½ minutes to 40 seconds and the coagulation time had dropped from 11½ minutes to 5 minutes. Throughout hospital stay prothrombin time and clotting time remained consistently low and on the seventh day prothrombin time was 24 seconds and clotting time was 3 minutes.

Prothrombin determinations were done according to the method of Quick.[1]

1 Quick, Armand J., Stanley-Brown, Margaret, and Bancroft, Frederic W., *Am. J. Med. Sci.*, 1935, **190**, 501.

Summary. In 2 infants an abnormally high prothrombin time and clotting time was strikingly reduced by administration of a concentrate rich in so-called Vitamin K. The dramatic results in 2 cases suggest that concentrates of this nature may prevent the occurrence of hemorrhagic disease of the newly-born and its tragic consequences.

10444 P

Comparative Effects of Sulfapyridine and Sulfanilamide in Type II Pneumococcic Infection of Mice.

GEORGE W. RAIZISS, M. SEVERAC, J. C. MOETSCH AND L. W. CLEMENCE.

From the Dermatological Research Laboratories, Philadelphia, Division of Abbott Laboratories, North Chicago, Ill.

Whitby[1] described a new derivative of sulfanilamide, M & B 693, or sulfapyridine, as being effective in both experimental pneumococcic and streptococcic infection in mice. Whitby found with Type I pneumococcic infection, that a dose of 10 mg of sulfapyridine given to mice by mouth permitted an average survival of 4.6 days. On the other hand, in a similar experiment, a dose of 10 mg of sulfanilamide gave an average survival time of 1.9 days.

Several clinicians have found this product effective in the treatment of human pneumonia due to pneumococci of Types I, II, and III.

Chemically, sulfapyridine is 2-(*p*-amino-benzene-sulfonyl)-aminopyridine. It is sparingly soluble in water at room temperature and somewhat more soluble on heating. The authors used a product prepared in their laboratories, which was purified by several recrystallizations from 50% alcohol and melted at 193-194°C. Analysis showed nitrogen, 16.4%; sulfur, 12.6%; theory, N, 16.8%; S, 12.8%. In addition, sulfapyridine obtained from commercial houses, which had the same chemical properties, was included in our studies.

The toxicity of sulfapyridine was studied on rabbits. Two g per kg of body weight administered *per os* were tolerated by 4 of 6 animals. We had found[2] that 2 g of sulfanilamide were tolerated by

1 Whitby, L. E. H., *Lancet*, 1938, 1210.

2 Raiziss, G. W., Severac, M., Moetsch, J. C., and Clemence, L. W., *J. Chemoth.*, 1938, 91.

50% of rabbits. While this indicates that sulfapyridine is tolerated a little better by rabbits, it must be considered that, being less soluble in water than sulfanilamide, it is absorbed less completely.

Therapeutic Effect. Mice were infected with 100 MLD of Type I, II, or III pneumococcus, whose average minimal lethal dose was 0.5 cc of 1:10,000,000 dilution of broth-culture. The drugs were given by mouth; the dose was 10 mg; the first was administered 1½ hours, the second 6 hours after infection. On the second day 2 doses were given and thereafter one daily. The maximal number of treatments was 10. Table I presents a summary of our experiments.

TABLE I.
Effects of Type II Pneumococcic Infection Followed by the Administration of Sulfanilamide or Sulfapyridine by Mouth.*

Drug	No. of mice used	% surviving after (days)							
		1	2	3	4	5	6	7	8
Sulfapyridine	50	100	98	56	26	18	12	10	8
Sulfanilamide	45	89	24	0	0	0	0	0	0
Controls	80	46	0	0	0	0	0	0	0

*The result of several individual experiments.

Conclusions. While sulfapyridine is somewhat more potent than sulfanilamide in the treatment of Type II pneumococcic infection in mice, it does not cure the animals, but only delays death. Untreated mice survived the infection for 1 day. Twenty-four percent of sulfanilamide-treated animals lived 2 days, while about an equal number of sulfapyridine-treated animals, namely 26%, survived 4 days. The superiority of sulfapyridine over sulfanilamide is only in the delay of death by 2 days. Similar experiments were made in Types I and III pneumococcic infection, and the results obtained were about the same as in Type II. In view of the reported good results observed with sulfapyridine in the treatment of pneumonia, our findings in experimental pneumococcic infection in mice are less encouraging than in human infection.

10445 P

Ultraviolet Irradiation of the Blood Stream in Septicemia.

Bennett F. Avery and Afif K. Mufarrij. (Introduced by E. W. Dennis.)

From the Department of Anatomy, American University of Beirut, Beirut, Lebanon.

Eidinow[1] found that irradiation of the skin of rabbits with a "daylight lamp" emitting rays from infra-red to 2850 Å for 48 hours previous to infection with staphylococci afforded considerable protection, but that identical irradiation immediately following infection was without effect. He also reported[2] that irradiation of defibrinated blood *in vitro* with rays from a mercury vapor lamp down to 2200 Å caused a gradual destruction of the bactericidal power, reaching a maximum in 1½-2 hours, whereas injection of a few cc of this irradiated material resulted in an increase in the bactericidal power of the blood withdrawn from the circulation 1-3 hours after the injection.

There have also been a number of studies[3] of various effects of irradiation of the blood circulating through a quartz cannula inserted in an artery. These demonstrated a massive fall in blood pressure, concentration of the blood, and changes in the relative concentrations of various constituents of the blood, following intensive and prolonged irradiation. The present investigation was undertaken to determine the influence of mild ultraviolet irradiation of the circulating blood of rabbits on the course of an established infection with *Salmonella typhimurium* and as a comparison with *Salmonella paratyphi.*

In all of the experiments, young rabbits of about 2 kg were used after having been isolated for a month to eliminate sickness or pregnancy. The controls were subjected to the same operative trauma and injected with equal amounts of the same bacterial suspensions as were the animals to be irradiated. The strain of *Salmonella typhimurium* which was used was obtained from Lister Institute, whereas the *Salmonella paratyphi* was a strain recently isolated in Beirut. For each experiment, one loopful of broth culture was transplanted to a tube of broth to be incubated for 24 hours at 37°C. One loopful of this broth was then planted on an agar slant and incubated. After

1 Eidinow, A., *Brit. Med. J.*, 1929, **2**, 293.

2 Eidinow, A., *J. Path. and Bact.*, 1930, **33**, 769.

3 Falk, I. S., and Reed, C. I., *Am. J. Physiol.*, 1926, **75**, 616; Reed, C. I., *Am. J. Physiol.*, 1925, **74**, 518, 525.

24 hours, the slant culture was suspended in normal saline. The amount injected was calculated to be fatal within 48 hours; in only 5 cases out of 58 did the control live longer and none survived (Table I).

TABLE I.
Effect of Irradiation of Infected Circulating Blood on Resistance of Rabbits.

	S. typhimurium		*P. paratyphi*	
	Irradiation	Control	Irradiation	Control
Total No. of rabbits used	36	30	48	28
No. surviving more than 48 hr	21 (58%)	4 (13%)	22 (46%)	1 (4%)
Avg survival time, in hr, of those which died	51	39	42	26
No. alive at end of 1 wk	3	0	5	0
Avg wt (g)	2069	2129	1917	2034

Under urethane anesthesia, the external jugular vein and common carotid artery were exposed and 10-15 mg of heparin per kilo of body weight was injected into the vein. This was followed by the fatal dose of bacteria. A system composed of a 45 mm quartz tube of 2 mm bore, connected at each end to glass cannulas of 1.2 mm bore by means of short rubber tubes, was then intercalated in the course of the artery. As soon as circulation through the quartz tube was observed to be satisfactory, irradiation was begun.

The source of light was a water-cooled Kromayer lamp operating at 4 amperes on 110 volts and giving between a first and a second degree erythema in 10 seconds' contact exposure. The irradiation time was varied from 4-15 minutes at 12-15 cm. Immediately after the irradiation the artery was ligated and the wound sutured.

The results given in Table I indicate that considerable protection is afforded to rabbits against otherwise fatal doses of either *Salmonella typhimurium* or *Salmonella paratyphi* by mild ultraviolet irradiation of the circulating blood, little difference being observed in the results obtained with the 2 organisms.

The maximum protection was obtained with 6 minutes' exposure at 12 cm. It should be stressed that the irradiation employed by us was very mild. Two rabbits subjected to 18 minutes at 15 cm (2-3 times the usual amount) suffered no observable ill effects and direct kymograph tracings of the blood pressure during the usual 6-8 minutes' irradiation at 15 cm showed no appreciable change. Furthermore, no sensitization seems to have occurred, since 2 of the rabbits were given second irradiations after a lapse of 2 hours with no harmful effects.

At least a part of the bacteria injected were present in the circulating blood at the time of irradiation, as shown by cultures taken from controls at 5 minutes, 2 hours, and 5 hours after the injection, all of which were strongly positive for the organism employed. Only a small fraction of the blood passing through the quartz tube at any one time could be reached by the ultraviolet rays, but the same blood passed repeatedly through the tube. We found that the total blood flow during 6 minutes' irradiation was about 600 cc, which is several times the total blood volume.

Autopsies were performed immediately after death and surviving animals were killed for autopsy after 2 weeks or more. None of the autopsies showed more than the usual variable sequelæ of the respective type of infection involved. All of the rabbits surviving more than one week showed persistently negative blood cultures.

In considering possible mechanisms producing the observed protection following irradiation of the blood stream, it should be noted that Guttmacher and Mayer[4] found that ultraviolet rays did not penetrate 1 mm of blood sufficiently to sterilize it. While it is possible that in our experiments sufficient bactericidal action did occur at the surface of the stream of blood passing through the quartz tube to influence the results obtained, the observations of Eidinow[2] on injection of irradiated blood, if confirmed, would suggest that the major effect was an indirect one, *i. e.*, the fraction of the blood which was reached by the ultraviolet rays entering the quartz tube may have acted like a small injection of irradiated blood in increasing the bactericidal power of the blood as a whole.

Summary. Mild ultraviolet irradiation of the circulating blood of rabbits which had been injected intravenously with doses of either *Salmonella typhimurium* or *Salmonella paratyphi* which were uniformly fatal to the controls resulted in a marked increase in survival time and in over 8% of the cases in recovery of the animal.

[4] Guttmacher, M., and Mayer, E., *Am. Rev. Tuberc.*, 1924, **10**, 170.

10446

Dried Skim Milk and Other Supplements in the Ration During Caecal Coccidiosis of Chicks.

ELERY R. BECKER AND P. C. WATERS.

From Iowa State College.

The unfavorable influence of dried buttermilk in the ration upon the course of experimental caecal coccidiosis of White Leghorn chicks has been adequately demonstrated by Becker and Waters[1] and Becker and Wilcke.[2] The first-named also made one test ("Experiment 4") comparing the effects of rations made up to 1% with dried skim milk, 6% with dried skim milk, and 6% with dried buttermilk, with results suggestive of an effect from skim milk like that from buttermilk, but not adequate for definite conclusions. Included in this report are further experiments with dry skim milk and buttermilk, and one designed to test the effect of substituting soy bean protein for part of the protein in meat scraps.

All chicks employed in these experiments were purchased from a commercial hatcheryman who conscientiously tests his laying flocks for pullorum disease and markets only vigorous day-old chicks. They were kept in metal brooders with mesh bottoms from the time they were purchased to the completion of the experiment. Immunizing or protective effect of previous infection was ruled out by occasional microscopic examination of caecal eliminations and constant watching of the droppings for traces of blood. Rations to be tested were fed for a period preliminary to the experimental infection, and the latter was accomplished by inoculating a determined-upon number of sporulated oöcysts of *Eimeria tenella* directly into the crop through a rubber catheter attached to a Luer syringe. Every chick that succumbed was examined postmortem to confirm death from caecal coccidiosis.

Experiment 1. The effects of 3 rations were compared: A, with 6 parts (dry weight) dry buttermilk; B, with 6 parts dry skim milk; C, without dried milk, but with 6 parts soy bean oil meal (expeller process) instead. These protein supplements were mixed as needed with the following stock ration whose parts add up to 94: ground yellow corn, 35; ground whole oats, 10; wheat bran, 5; wheat middlings (gray shorts), 30; alfalfa meal, 13% protein, 2; fish meal (sardine), 6; steamed bone meal, 1; fine oyster shell, 2; salt, 1; char-

1 Becker, Elery R., and Waters, Philip C., *Ia. St. Col. J. Sci.*, 1938, **12**, 405.

2 Becker, E. R., and Wilcke, H. L., *Poul. Sci.*, 1938, **17**, 405.

coal, 1; cod liver oil, 1. The estimated total protein of rations A, B, and C, was 16.4%, 16.4%, and 16.8%, respectively.

The White Leghorn chicks were started on the rations at the age of 5 days after a preliminary period of 4 days on a commercial ration. At the end of a 17-day feeding period, or at the age of 22 days, they were inoculated with 50,000 oöcysts per bird. The average weight gains for the feeding period to the fourth day of the infection, by which time there are ordinarily few symptoms of the disease, were as follows: A, 167 g; B, 140 g; C, 145 g. Fatalities for the 3 rations, which occurred on the fifth and sixth days of the infection, were as follows: A, 11 out of 29 chicks; B, 10 out of 28 chicks; C, 3 out of 28 chicks. The birds were kept for 10 days after the last fatality (on the sixth day) without further losses. The weight gains for the survivors over a 30-day period, which included the period of illness, were as follows: A, 257 g; B, 236 g; C, 234 g.

It is evident that the losses for both dried milks were appreciably greater than those for the soy bean oil meal, or lack of dry milk.

Experiment 2. A ration (D) with 6 parts dry skim milk was compared with 3 rations (E, F, G) with drastically reduced milk content. Since 3 cases of partial paralysis had appeared among chicks on ration C, 1 part of dry skim milk was used to forestall its recurrence. The supplements were as follows: (E) skim milk 1, casein (fine, commercial) 2, ground yellow corn 3; (F) skim milk 1, soy bean oil meal (expeller process) 4, casein 1; (G) skim milk 1, fish meal 3, ground yellow corn 2. Each ration was made by mixing 6 parts of appropriate supplement with 94 parts of the following stock ration: yellow corn meal, 41.5; ground whole oats, 10; wheat middlings (gray shorts), 25; bran, 5; alfalfa meal (13% protein), 2; soy bean oil meal, 1; meat scraps, 2; sardine meal, low temperature, 2; steamed bone meal, 0.75; fine limestone, 2; fine charcoal, 1; salt, 0.75; cod liver oil, 1. The estimated protein content of rations D, E, F, and G was 15.0, 15.3, 15.8, and 15.5, respectively.

Four-day-old White Leghorns were started on the 4 rations, and inoculated with doses of 185,000 oöcysts at the age of 27 days. Mean weight gains for the 4 lots to the fourth day of infection were as follows: D, 195.6 g; E, 203.7 g; F, 213.8 g; G, 185.7 g. Mortality records: D, 16 out of 29 chicks; E, 9 out of 27; F, 10 out of 29; G, 6 out of 18. If the chi-square method is used to compare mortality data for recipients of 6 parts skim milk (D) with total recipients of 1 part skim milk (E, F, G), a significant difference is indicated.

Weight gains from the fourth to the twelfth day of the infection,

which included the fifth and sixth days on which fatalities occurred, were (D) 60 g, (E) 66 g, (F) 67 g, and (G) 62 g. These records are averages based on individual weighings of survivors. They indicate no essential difference in the rate of recovery.

Experiment 3. The writers' experiments have shown a more favorable prognosis in caecal coccidiosis when soy bean oil meal is substituted for a considerable part of the customary dry milk concentrate in the ration. This experiment and the next were planned to test the effect of substituting soy bean oil meal for part of the dried meat element in meat scraps, and a special low-temperature fish meal for the dry milks.

There were 4 lots of White Leghorn chicks: L1 and L2 on a ration (I) consisting of meat scraps, 8 pt, steamed bone meal, 1 pt, soy bean oil meal, 5 pt, dried skim milk, 5 pt, and other ingredients in somewhat the same proportions as in Experiment 2; L3 and L4 on a ration (J) with about the same composition, except that meat scraps were reduced to 2 parts, soy bean oil meal was raised to 11 parts, and steamed bone meal to 3 parts. The total protein content of I was 17.5%; of J, 16.7%. The chicks were put on the special rations at the age of 7 days, and infected at the age of 21 days. L1 and L3 received 100,000 oöcysts per chick; L2 and L4, 200,000 oöcysts per chick.

The mortality for the 4 lots was as follows: L1, 12 out of 31 chicks; L2, 14 out of 32; L3, 14 out of 33; L4, 13 out of 30. Thus, there was no significant difference between the losses in the 4 lots. Weight gains were recorded, but they too differ little.

Experiment 4. The stock ration used for these tests was, in general, like that in Experiment 2, except that middlings was reduced to 8 parts and ground oats raised to 21 parts. Ration K contained 6.5 pt dried buttermilk; L, 6.5 pt dried skim milk; M, 6 pt special low-temperature sardine meal from the Pacific Coast. The chicks were put on the rations at the age of 6 days, and infected with doses of 50,000 oöcysts at the age of 3 weeks. The losses on the 3 rations were as follows: K, 10 out of 29 chicks; L, 12 out of 30; M, 11 out of 30. Thus, the 3 lots suffered about the same and the rations were without differential effect. Likewise, weight gains were not appreciably different.

Summary. Further evidence is supplied for the unfavorable effect of dry buttermilk in the ordinary type of growing ration when White Leghorn chicks become infected with sizable doses of *Eimeria tenella*. It is now shown that dry skim milk has an effect similar to that of dry buttermilk. It made little difference when soy bean oil

meal (expeller process) was substituted for a considerable part of the meat element in meat scraps, or when a fine grade of low-temperature sardine meal was substituted for dry milks.

Further, it was brought out in chicks on 2 different rations that there was no difference in mortality from infective inoculations of 100,000 and 200,000 oöcysts (Experiment 3).

10447

Tests for the Eye-Color Hormones of Drosophila in Other Insects.

L. W. LAW.* (Introduced by C. C. Little.)

From the School of Biological Sciences, Stanford University.

Diffusible substances hormone-like in nature are concerned in the eye-color development of Drosophila[1] and certain other insects (Ephestia,[2] Bombyx,[3] and Habrobracon[4]). It has been shown that the substances in Ephestia, Habrobracon and Drosophila are not species-specific.[4] Many of the chemical properties of these substances have been determined.[5, 6] In Drosophila they are active when injected (extract form) into,[7] or fed to,[8] appropriate test larvae. The v^+ substance brings about a modification of the eye color of vermilion brown *(v bw)* test larvae from a pale pink towards brown, and the cn^+ substance induces a similar modification of cinnabar brown *(cn bw)* eye color from colorless towards brown.

A question of interest in regard to the nature of these hormone-like substances is: Are they normal constituents of tissue; are they restricted to certain insect groups, or is their occurrence general throughout the class Insecta? It is the purpose of this paper to pre-

* Parker Fellow from Harvard University, 1937-38.

1 For review of literature see Ephrussi, B., *Am. Naturalist*, 1938, **72**, 5.

2 Kühn, A., *Z. indukt. Abst. u. Verer.*, 1937, **73**, 419.

3 Kikkowa, H., *Zoöl. Mag.* (Japan), 1937, **49**, 348.

4 Beadle, G. W., Anderson, R. L., and Maxwell, Jane, *Proc. Nat. Acad. Sci.*, 1938, **24**, 80.

5 Khouvine, Y., and Ephrussi, B., *Compt. Rend. Soc. Biol.*, 1937, **124**, 885.

6 Tatum, E. L., and Beadle, G. W., *J. Gen. Physiol.*, 1938, **22**, 239.

7 Beadle, G. W., Clancy, C. W., and Ephrussi, B., *Proc. Roy. Soc. B*, 1937, **122**, 98.

8 Beadle, G. W., and Law, L. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 621.

sent data on feeding tests made on tissues of representatives of most of the insect orders.

A few tests have been made for these substances in other organisms. Khouvine and Ephrussi were unable to demonstrate any hormonal activity in nitrogenous extracts of sheep brain. The author was unable to detect any activity in embryonic tissues of mice by both the injection and feeding technics.

The insects tested were collected in larval and pupal stages and identified in nearly all cases as to family. Where possible several growth stages have been included in a test to be sure of detecting any hormonal activity. The tissues were placed in boiling H_2O for 30 to 40 seconds to inactivate the enzyme, dried in the oven for ½ hour at 100°C, and crushed in a few drops of Ringer's. This material was then placed in vials on a 1% agar, 0.25% yeast medium.

Eggs from which the *v bw* and *cn bw* test larvae were obtained were collected over a 2-hour period at 25° and the larvae were grown on a standard cornmeal-molasses-agar medium seeded with yeast. The test larvae were removed from the food at approximately 75-84 hours after egg laying and transferred to the vials. In this manner there can result no change in the eye color due to "starvation effect".[9] From 10 to 15 larvae were used in each test. The resulting responses in the adult Drosophila eyes have been graded according to a standard color scale.[6]

Feeding tests were first made using representatives of the 3 orders known to possess the v^+ and cn^+ hormones. In the Diptera Chaoboris and Culex (Culcidae) and a Chironomid midge (Chironomidae) gave strong positive tests for both substances. In addition to Bombyx and Ephestia 2 other Lepidoptera have been tested, Vanessa and Pseudohazis. Both gave relatively strong tests. Four additional families were tested in the Hymenoptera, Formicoidae (ant), Bombidae (bumble-bee), Tenthridinidae (saw fly) and Apidae (honey-bee and wasp). There was found to be no influence on the eye color of test larvae by tissues of the ant, bumble-bee or honey-bee. However, tissues of the wasp (also Apidae) produced a decided color change of 0.8 units in *v bw* and 0.5 units in *cn bw* animals, and tissues of the saw fly produced a comparable modification. All tests giving negative results were repeated 2 or 3 times.

In addition to these 3 orders, representatives of 10 other insect orders have been tested (Table I). Modifications were obtained in all orders except Isoptera, where only one family was available for study at the time. The greatest modification in eye color was pro-

[9] Beadle, G. W., Tatum, E. L., and Clancy, C. W., *Biol. Bull.*, 1938, **75**, 447.

TABLE I.

Effect of Feeding Crushed Tissues of Various Insects on the Eye-color of Vermilion Brown (*v bw*) and Cinnabar Brown (*cn bw*) Larvæ.

Insects used	*v bw* test animals		*cn bw* test animals	
	No.	Color value[6] (mean and range)	No.	Color value (mean and range)
Trichoptera*				
Leptoceridæ				
caddis fly	10	0.85 (0.5-1.0)	10	0.56 (0.5-0.7)
Plecoptera*				
stone fly	11	0.34 (0.2-0.5)	6	0.20 (0.1-0.4)
Orthoptera*				
Acridiidæ				
grasshopper	10	2.7 (2.0-3.0)	8	0.95 (0.8-1.0)
Gryllidæ				
Jerusalem cricket	14	0.0	11	0.0
Hemiptera*				
Aphididæ				
plant lice	8	1.6 (1.0-3.0)	8	0.8 (0.7-1.0)
Corixidæ				
water boatman	10	2.3 (2.0-3.0)	12	2.3 (1.5-3.0)
Dytiscidæ				
giant water beetle	10	0.5 (0.0-0.7)	11	slight
nymphal Hem.	11	1.3 (1.0-2.0)	9	1.2 (1.0-1.5)
Odonata				
Lestidæ				
dragon fly	11	0.7 (0.5-1.0)	16	0.62 (0.5-1.0)
Isoptera				
Termitidæ				
termites	14	0.0	10	0.0
Ephemeroptera				
may fly	12	1.3 (0.5-2.0)	9	1.3 (1.0-1.5)
Thysanura*				
Machilidæ				
fish moth	7	1.6 (1.0-2.0)	9	1.4 (1.0-2.0)
Neuroptera				
Raphidioidæ				
Raphidia	9	2.2 (2.0-3.0)		
Myrmeleonidæ				
ant lion	10	0.96 (0.5-1.5)	6	2.0
Coleoptera				
Bruchidæ				
bean weevil	15	0.0	12	0.0
Coccinelidæ*				
lady beetle				
C. bipunctata	12	2.2 (2.0-2.5)	12	1.1 (0.5-1.5)
Dermestidæ*				
Dermestis lardarius	13	0.61 (0.5-1.0)	12	slight
Chrysomelidæ				
tortoise beetle	13	1.9 (1.5-2.5)	12	1.2 (0.5-1.5)
Controls				
1% agar; 0.25% yeast	19	0.0	13	0.0

*Cross-comparable.

duced by tissue of the grasshopper (Acridiidae) and by *Coccinella bipunctata*. However, in both these orders there were found insects of other families which produced no modification whatsoever. No

explanation can be given for these results at the present time.

In every case where there was obtained a test for v^+ substance there was also obtained a test for cn^+ substance, giving further indication of their close chemical relationship. Likewise, in all tests which were cross comparable, there always resulted a greater modification of *v bw* eyes than of *cn bw* eyes.

Acknowledgment is made to Mr. Leigh Chadwick of Harvard University for supplying some of the insects used in this test.

Summary. The diffusible substances concerned in eye color development in Drosophila are widely distributed throughout the insect group. They are probably normal constituents of insect tissues only, since tests in other animals have been entirely negative.

Tissues producing a modification of *v bw* eyes also produce a modification of *cn bw* eyes. In all cross comparable cases there has resulted a greater modification of *v bw* eyes than of *cn bw* eyes.

10448 P

Depression of Polycythemia by Choline Hydrochloride.

JOHN EMERSON DAVIS. (Introduced by C. S. Leonard.)

From the Departments of Pharmacology and Biochemistry, University of Vermont, Burlington, Vermont.

The author[1] presented work which showed that the feeding of raw beef or hog liver to polycythemic (experimental) dogs caused a prompt reduction in their erythrocyte numbers, by depressing hemopoiesis.

Recently, Jacobs[2] has reported that liver extract, prepared for oral use, contains at least 1% of choline.

This report led us to the investigation of the effect of orally administered choline hydrochloride upon polycythemic dogs. Polycythemia was produced experimentally in 2 dogs by placing them for 6 hours daily in a low pressure chamber in which the environmental air pressure was reduced to about 430 mm of mercury by a motor driven vacuum pump. Within 10 days, this procedure had induced consistent increases (27 and 30%) in the erythrocyte numbers of the animals when in an approximately basal state. The hemoglobin percentage (Sahli) was increased correspondingly and reticulocytosis occurred.

1 Davis, J. E., *Am. J. Physiol.*, 1938, **122**, 397.

2 Jacobs, H. R., *J. Lab. and Clin. Med.*, 1938, **24**, 128.

When 100 mg of choline hydrochloride per day was administered orally, prompt reductions (of 15 and 20%) in the red cell counts occurred within 3 days, in spite of daily exposure of the animals to low atmospheric pressure. Reticulocyte percentages were also reduced, but the total leukocyte counts did not change significantly. Upon cessation of choline feeding, the erythrocyte numbers returned to their polycythemic values.

In 2 other dogs, the erythrocyte numbers were increased by 15% by daily oral administration of 8 mg of cobalt chloride per kg of body weight, over periods of 8 to 10 days (for details of method see reference to the author[3]). Upon feeding 8 mg of choline hydrochloride per kg of body weight per day, in 1% solution by stomach tube, in addition to cobalt, there was a prompt reduction of the erythrocyte numbers to 4 and 6% below normal in 3 days. The reductions in erythrocyte numbers, reticulocyte and hemoglobin percentages persisted for 6 days during which choline was fed. After discontinuation of the choline, these values gradually increased, over a period of 5 days, to their polycythemic values. The total leukocyte counts did not change significantly throughout these experiments.

The same dose of choline (8 mg per kg) was administered to 2 normal dogs over a period of 6 days, and did *not* change their normal erythrocyte numbers significantly.

These experiments indicate that the oral administration of choline hydrochloride depresses hematopoiesis in polycythemic dogs, and tends to return the red cell number to normal.

10449

Effect of Certain Posterior Hypophyseal Extracts on Ciliary Motion.

AUSTIN E. SMITH. (Introduced by E. M. Boyd.)

From the Department of Pharmacology, Queen's University, Canada.

This report deals with an attempt to ascertain the effect of certain pituitary extracts on the rate of ciliary movement of the esophageal and pharyngeal mucosa of healthy frogs *(Rana pipiens)*, weighing 25 g each. The brain and spinal cord were carefully pithed, the lower jaw removed, and the esophagus and stomach exposed by cutting through the pectoral girdle. The pharynx and esophagus

[3] Davis, J. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 96.

were then incised ventrally. The frog was then pinned in a supine position without stretching the area to be observed. Mucus was removed gently by stroking with absorbent cotton saturated with frog saline.

The stimulation of ciliary movement was evaluated by determining the interval during which a mustard seed was transported a constant distance. Fourteen to 20 control determinations preceded the testing of any of the substances. All experiments were conducted at the same room temperature. All the solutions and the frog saline were added at the same rate and the same volume through a pipette. Recovery time was noted in every experiment; the cilia were not rendered permanently non-motile in any experiment.

Pituitrin* was the first solution added. Its effect on passage time was recorded at 1, 2 and 3 minute intervals. Pitocin and pitressin were then applied and any comparable results compiled. Forty-five frogs were used in approximately 360 experiments, each result being the average rate of passage of many mustard seeds. Various dilutions of acetic acid were also applied to eliminate the acidity of the pituitrin as a ciliary stimulant.

TABLE I.
% Increase of Ciliary Motion After Topical Application of Pituitrin.

% concentration	% increase of ciliary motion after			% experiments showing stimulation
	1 min.	2 min.	3 min.	
2.0	23.3	30.7	32.0	100.0
1.0	20.0	26.1	22.6	88.8
0.2	15.2	24.6	18.9	63.6
0.1	16.5	5.3	2.4	70.0

Table I illustrates the increased motility and its prolongation induced by the topical application of certain concentrations of pituitrin extracts applied to pharyngeal and esophageal mucosa of the frog.

TABLE II.
% Increase of Ciliary Motion After Topical Application of Pituitrin, Pitocin, Pitressin and Acetic Acid.

Substance	%	% increase of ciliary motion after application
Pituitrin	2.0	12.2
Pitocin	2.0	17.6
Pitressin	2.0	14.2
Acetic acid	0.02	—20.2 (decrease)
" "	0.5	cilia motionless

* The Pituitrin (Surgical) was generously donated to the Department of Pharmacology by Dr. E. A. Sharp of Parke, Davis and Co.

Table II indicates that pitocin stimulated ciliary activity more than the other substances tested and that acetic acid depressed and finally arrested ciliary activity. Thus the stimulating effect of pituitrin is apparently due to some property of the extracted glandular material other than its acidity.

10450 P

Urinary Concretions Caused by Sulfapyridine.

PAUL GROSS, FRANK B. COOPER AND MARION LEWIS.

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Pa.

Sulfapyridine is being widely used in clinical experiments because of its alleged lack of toxicity and the promising results reported by Whitby,[1] and by Evans and Gaisford.[2] However, our investigations,[3, 4] as well as those of others,[5, 6] have failed to confirm Whitby's claims that the drug is capable of protecting mice against 10,000 fatal doses of pneumococci; and we have pointed out that the order of efficacy of this drug and of sulfanilamide is approximately the same in rats. Marshall, Bratton and Litchfield[7] have recently issued a warning in regard to the erratic absorption and the potential toxicity of the drug, stating that sulfapyridine should not be used where sulfanilamide is indicated. Long and Finestone[6] have also drawn attention to the fact that the absorption and excretion of sulfapyridine are erratic.

In the course of an investigation concerning the delayed deaths of rats treated with sulfapyridine, we noted that although the infections were in some instances apparently cured, the kidneys were enlarged and soft, the pelves and ureters dilated, and the bladders empty and contracted. Careful examination of the urinary tract revealed crystalline, spiculated, white or yellow concretions and sand, often im-

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Evans, G. M., and Gaisford, W. F., *Lancet*, 1938, **2**, 14.

3 Cooper, F. B., Gross, P., and Lewis, M. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 37.

4 Cooper, F. B., Gross, P., and Lewis, M., to be published.

5 Hilles, C., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 73.

6 Long, P. H., Bliss, E. A., and Finestone, W. H., *Pennsylvania M. J.*, 1939, **42**, 483.

7 Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., Jr., *Science*, 1939, **88**, 597.

pacted, with more or less complete urinary obstruction. A chemical analysis of these concretions indicated 6.4% sulfapyridine and 64.1% acetylsulfapyridine. These findings were absent in untreated rats, and in rats treated with sulfanilamide; all of which had been on the same diet. The dosage employed was the same for both drugs: one-half to one gram per kilo per day for 8 to 14 days.

Higgins[8] has produced urinary calculi in rats with vitamin A deficient diet. The calculi described by Higgins were spherical and light brown, consisted chiefly of calcium phosphate, and were associated with alkaline urine. The diet of our rats consisted of fresh carrots, dog biscuits (said to contain vitamins A and D), oats, and white bread. The urine of normal rats fed on this diet has a pH 5.5 to 6.5. The urine of the rats with calculi had a similar range.

The possibility must not be overlooked that similar concretions with necessarily delayed symptomatology may develop in man as a result of sulfapyridine administration.

10451 P

Experimental Vascular Disease in Rats Produced by Multiple Depletions of Vitamin A.*

LINCOLN OPPER. (Introduced by Barnett Sure.)

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.

The pathology of chronic vitamin deficiency is usually studied in laboratory animals which have been subjected to a partial deficiency over a long period of time. This dietary method has been considered to represent the closest analogy to the course of the human disease.[1] The present experiment was designed to effect pathologic changes in the rat by successive and complete depletions of vitamin A rather than by sustained and partial withdrawal of the vitamin. To obtain this end, multiple avitaminotic episodes of varied severity were interspersed with periods of relative recovery.

Procedure. Twenty-four pairs of rats, litter-mates of the same

8 Higgins, C. C., *J. A. M. A.*, 1935, **104**, 1296.

* Research paper No. 619, Journal Series, University of Arkansas. Aided in part by a grant from the Committee on Scientific Research of the American Medical Association.

1 Editorial, *J. A. M. A.*, 1938, **111**, 2019.

sex and ranging in weight from 50 to 100 g, were given a basal ration† deficient in vitamin A and supplemented only in the control rat of each pair with 3 drops of carotene in oil.‡

Successive depletions in each pathologic rat were usually terminated by the oral administration of carotene. This vitamin was provided only until the fall in weight was definitely checked. Diarrhea sometimes made necessary the subcutaneous injection of 0.5 cc of cod liver oil for a brief period. Response to the resumption of vitamin therapy varied in each animal with each depletion, due apparently to variations in degree of the underlying pathology, chiefly of the renal or of the respiratory tract. Two or 3 depletions were obtainable in each rat during an experimental period ranging from 127 to 238 days. For histologic study of the arterial system, the tissues following autopsy were fixed in Bouin's solution, embedded in paraffin and stained with hematoxylin-eosin or v. Kossa's method for calcium.

Vascular Pathology. All but 3 of the 24 multiply depleted rats showed some degree of vascular injury. No trace of morphologic change was observed in the vessels of any of the 24 control rats. The majority of the involved aortas were characterized grossly by loss of elasticity, dilatation and the presence of firm, intramural plaques. The major branches of the aorta revealed marked tortuosity. Histologically the changes were in the nature of a degenerative medial process which affected practically the entire arterial system down to the larger arterioles. Arteries within the stroma of the liver, kidney and spleen, however, were invariably spared. Necrosis of the smooth muscle cells of the media was accompanied by a deposition of calcific granules in pericellular and perilamellar distribution. This was followed by the formation of large calcium plaques and the growth of granulation tissue in the media, and by a proliferation of fibrous connective tissue in the subintima. The latter sometimes led to the complete closure of small visceral arteries. When this took place in the heart, myocardial fibrosis was the result.

The pathogenesis of the arterial injury is uncertain. If the lesions are due directly to the avitaminosis, this represents the first instance of primary pathology in tissues other than those of epithelial origin. Before this interpretation may be accepted, however, further studies of the secondary effects of chronic vitamin A deficiency are necessary.

† Casein, 20; McCollum's salts No. 185, 4; Northwestern yeast (dehydrated), 10; Dextrin, 51; Crisco, 15. The ration was irradiated for 30 minutes to provide vitamin D.

‡ Generously supplied by S. M. A. Corporation, Cleveland, Ohio.

10452

Distribution of Sulfanilamide and Acetylsulfanilamide Between Cells and Extracellular Fluid.*

HERBERT S. SISE. (Introduced by Soma Weiss.)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Since sulfanilamide when given to dogs has been recovered in all organs in concentrations proportional to the water contents thereof,[1] it has been suggested that this drug might offer a convenient means of estimating total body water. A method which involves the same principles as those employed in the dye technic for estimation of plasma volume[2] has been used to estimate the total body water in dogs.[3] The results indicated that sulfanilamide was dissolved in approximately the same amount of water as urea, which was administered simultaneously; and, since urea is known to be distributed uniformly in body water, the sulfanilamide method seemed applicable for dogs. In expectation that this method might apply to man as well as dogs, the following experiments were carried out. These were done in the hope that the acetylation of sulfanilamide, a process which, although absent in dogs, is normally present in man,[4, 5] might not interfere with the determination.

Four subjects in basal conditions received an intravenous injection of 40 cc of a 700 mg % solution of sulfanilamide in 0.85% saline. Two additional subjects received intravenous injections of 100 cc of a 100 mg % solution of acetylsulfanilamide.† Samples of blood for control determinations were taken before the injection, and subsequent samples were taken in bottles containing the appropriate amount of potassium oxalate (2 mg per cc of whole blood) at varying intervals following the injection. The whole blood was analyzed for both free and total sulfanilamide by the revised method of Mar-

* The author wishes to express appreciation for the help and advice of Drs. Wan-nien Bien and Florence Haynes.

1 Marshall, E. K., Emerson, K., and Cutting, W. C., *J. Pharm. and Exp. Therap.*, 1937, **61**, 196.

2 Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 301.

3 Painter, E. E., *Proc. Am. Phys. Soc., Am. J. Phys.*, 1938, **123**, 159.

4 Marshall, E. K., Emerson, K., and Cutting, W. C., *J. A. M. A.*, 1937, **108**, 953.

5 Marshall, E. K., Cutting, W. C., and Emerson, K., *Science*, 1937, **85**, 202.

† Supplied by Winthrop Chemical Company.

shall,[6] and the final color reaction was read on an Evelyn Photoelectric colorimeter.

The resulting figures showed that in 3 of the 4 subjects who received sulfanilamide there were gross irregularities in the disappearance curves due to a 10 to 30% rise in the concentration of the total sulfanilamide in the blood occurring from 1½ to 6 hours after the injection. The concentration of the free form alone, on the other hand, showed a fairly smooth logarithmic type of disappearance curve. By subtracting the free from the total, the concentration of the acetyl form was obtained. The rise in the total concentration was thus found to coincide with the appearance and sudden rise in the concentration of the acetylated form. In the other subject, where there was no rise in the total concentration, there was no acetylsulfanilamide formed before the experiment was stopped.

When the acetylsulfanilamide was injected, there was a smooth logarithmic type of disappearance curve without any rise by the end of 6 hours; but the concentration was many times higher than would be expected assuming the drug to be equally distributed in total body water. In one subject there was no free sulfanilamide found at the end of 6 hours, and in the other a trace was detectable at 6 hours.

In order to determine the distribution of sulfanilamide and acetylsulfanilamide between the whole blood and plasma, simultaneous whole blood and plasma determinations of free sulfanilamide were carried out on 4 blood samples from 2 patients who received intravenous injections of sulfanilamide. Also 5 samples from the 2 subjects mentioned above, who received acetylsulfanilamide intravenously, were analyzed for the whole blood and plasma concentrations of the free and total fractions. The water content of the whole blood and plasma of these subjects was determined by placing accurately weighed and measured 0.5 cc samples in an oven at 37°C and continuing the drying process in a desiccator at room temperature until constant weight was reached. The results, shown in Tables I and II,

TABLE I.
Distribution of Sulfanilamide Between Plasma and Whole Blood *in Vivo*.

Subject	Whole Blood Concentration mg%	Plasma Concentration mg%	Water Content Whole Blood % by volume	Water Content Plasma % by volume
A.M.	.792	.364	78.6	92.6
	.549	.218		
E.M.	1.89	.616	83.0	92.4
	.687	.383		

[6] Marshall, E. K., and Litchfield, J. T., *Science*, 1938, **88**, 85.

TABLE II.
Distribution of Acetylsulfanilamide Between Plasma and Whole Blood *in Vivo.*

Subject	Whole Blood Concentration mg%	Plasma Concentration mg%	Water Content Whole Blood % by volume	Water Content Plasma % by volume
J.M.	.763	0	84.4	92.6
	.630	0		
	.578	0		
J.P.	.546	0.157	84.2	93.3
	.180	0.064		

indicated that a larger amount of free sulfanilamide was found in whole blood than in plasma; whereas this ratio was not anticipated from a comparison of their relative water contents. Almost all of the acetyl compound was found in the whole blood. These results indicate that these compounds are affixed to the blood cells in some form of loose combination, and that the acetyl compound is combined in relatively larger amounts than the free sulfanilamide.

In order to confirm the results *in vivo* by experiments *in vitro,* known amounts of sulfanilamide and acetylsulfanilamide were added to human blood samples, and the whole blood concentrations, plasma concentrations, whole blood water content, and the plasma water content were determined as above. These experiments showed that both sulfanilamide and acetylsulfanilamide were distributed between plasma and blood cells in the same proportion as in the experiments above, and that at high concentrations a smaller percent of the added amount was combined than at low concentrations. The results of experiments in which the concentration of sulfanilamide was kept constant while the concentration of red cells in saline suspension was varied are shown in Table III. There is some indication to believe that sulfanilamide combines with other body cells since in one of the cases in which acetylsulfanilamide was injected there was none in the plasma, which is considered to be in equilibrium with the extra-

TABLE III.
Effect of Varying the Volume of Red Cells in Saline Suspensions on the Concentration of Sulfanilamide in the Suspending Fluid When Sulfanilamide Is Added to Produce a Concentration of 33.3 mg per 100 cc of Suspension in All Experiments.

Volume Red Blood Cells %	Concentration of Sulfanilamide in Suspending Fluid mg%
0.0	33.3
11.9	31.1
23.8	29.4
35.7	28.3
47.6	26.2

cellular fluid. By determining the theoretical whole blood concentration at the moment of injection by means of extrapolating the disappearance curve back to the zero time, and from estimating the total blood volume, according to Gibson and Evans' values[7] for normal subjects, it was calculated that approximately 44% of the acetylsulfanilamide was carried in the blood cells of this individual. Since at the zero time none had been excreted and none had been changed from the acetyl form to the free form, and since none was found to be in the extracellular fluid, as represented by the plasma, the other 56% was presumably combined with other cells of the body.

It is concluded that sulfanilamide and acetylsulfanilamide in man are not distributed within the blood cells and plasma in relation to the water content. Sulfanilamide has an affinity for the blood cells while acetylsulfanilamide has an even more marked affinity for these cells. There is some indication for believing that these drugs also combine in small amounts with other cells in the body.

10453 P

Does Acetylcholine Play a Part in the Mechanism of Melanophores Expansion?

YIN-CH'ANG CHIN. (Introduced by Hsi-chun Chang.)

From the Department of Physiology, Peiping Union Medical College, and the Department of Biology, Yenching University, Peiping.

The action of acetylcholine (AC) on the melanophores was tested by Parker[1, 2] on the Fundulus and Ameiurus. When he found in 1931 that AC, unprotected by physostigmine, caused *dispersion* of the melanophore pigment in fairly large dose, he concluded that AC does not play a part in its normal control. And when he found in 1934 that AC, protected by physostigmine, caused a slight *concentration* of the melanophore pigment in fairly large amount, he made the same conclusion. Recent experiments done on the paradise fish *(Macropodus opercularis)* suggested, however, that AC may be involved in the mechanism of melanophore expansion in this species.

It was consistently demonstrated that 0.01γ AC chloride (E. Merck) injected into the body subcutaneously could produce a local

[7] Gibson, J. G., and Evans, W. A., *J. Clin. Invest.*, 1937, **16**, 317.

[1] Parker, G. H., *Proc. Nat. Acad. Sci.*, 1931, **17**, 596.

[2] Parker, G. H., *Proc. Nat. Acad. Sci.*, 1934, **20**, 596.

black area of 8x8 sq mm within 30-45 sec. after the injection, lasting for 10-13 min. Under binocular microscope, the melanophores were found to be expanded.

A purified extract was made from the caudal fins according to the method of Chang, Hsieh, Lee, Li and Lim[3] and was tested on toad's rectus and heart, and leech, (*Whitmania acranulata,* Whitman). AC was identified by the ratio between the unknown and the AC-standard before and after eserine on the rectus (Chang and Gaddum[4]), the atropine test on the heart, and the acid-alkali test on the leech. The extract was found to contain 0.5-1.0γ AC (as chloride) per g wet tissue.

Injection of an extract equivalent to 0.009γ AC could produce blackness in the caudal fin comparable to that produced by an equal amount of the AC-standard.

10454 P

Light-Pituitary Reflex and the Adrenergic-Cholinergic Sympathetic Nerve in a Teleost.

HSI-CHUN CHANG, WEI-MING HSIEH AND YUN-MING LU.

From the Department of Physiology, Peiping Union Medical College, Peiping.

Working on the snake fish (*Ophiocephalus argus,* Cantor), we found that its melanophores are under two types of control, one through the pituitary which is regulated by light and darkness, and the other through the sympathetic nerve which is partly adrenergic and partly cholinergic.

The Pituitary Control. After removal of the sympathetic chains at the level between the pectoral fin and cloaca, the fish developed, on a white background under the continuous illumination from a lamp of 100 W, a black color over the anterior denervated region. Subsequent enucleation of the eyes intensified this color into coal-black, whereas a previous hypophysectomy would prevent such an appearance. These results indicate clearly that the black color is due to the expansion of the melanophores produced by the pituitary principle whose continuous discharge is increased by darkness,[1] and

3 Chang, H. C., Hsieh, W. M., Lee, L. Y., Li, T. H., and Lim, R. K. S., in press.

4 Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

1 Jores, A., *Klin. Wchsch.*, 1935, **14**, 1713; Stutinsky, F., *C. R. soc. Biol.*, 1938, **127**, 409.

whose effect is accentuated in the absence of the sympathetic influence. Similar results were obtained from frogs *(Rana nigromaculata)*.

The Sympathetic Control. Adrenergic fibers. When the denervated area had become sufficiently sensitized, usually 3 days after the abdominal sympathectomy, stimulation of the myelencephalic sympathetic center would turn the black denervated area pale. From 10 days onward, the same phenomenon would occur spontaneously under continuous light illumination. This pallor is apparently due to diffusion into the denervated area of adrenaline-like substance liberated at the nerve-endings of the neighboring normal region in response to electrical or light stimulation. We may recall that Parker[3] has advanced similar explanation for the fading of the dark caudal band in *Fundulus*.

Cholinergic fibers. It was shown by Chin[2] that the paradise fish responds to very small dose of acetylcholine (AC) and that there is enough AC in the caudal fin to suggest for it a physiological rôle. Furthermore, we found that local mechanical stimulation such as cutting of the fin-rays could produce a dark band peripheral to the cut in the normal fish kept under light illumination, in keeping with the results of the earlier workers.[3] This phenomenon could also be shown in the hypophysectomized fish. On the other hand, we found that when nerve degeneration of the fins had well advanced after removal of the abdominal sympathetic chains similar cutting failed to produce the dark band, and the AC-content of the fin extract was markedly decreased, ranging from 1/6 to 1/2 of the normal, which was 0.077 γ/g. These results were confirmed on the paradise fish.

It may thus be concluded that the melanophores of the snake fish (possibly the paradise fish also) are innervated anatomically by the sympathetic nerve, but some of its fibers, which cause their dispersion, are mediated by AC, while the others, which cause their concentration, are mediated by adrenaline.

---

2 Chin Yin-ch'ang, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 454.

3 Wyman, L. C., *J. Exp. Zool.*, 1924, **39**, 73; Mills, S. M., *J. Exp. Zool.*, 1932, **64**, 231; Parker, G. H., Humoral agents in nervous activity with special reference to chromatophores, Cambridge Univ. Press, 1932; Color changes in animals in relation to nervous activity, Univ. Pennsylvania Press, 1936.

10455

Sensitization of Guinea Pigs to Cotton Linters and House Dust Extracts.

E. J. Coulson and Henry Stevens. (Introduced by F. A. Csonka.)

From the Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

Since Cooke[1] first directed attention to the importance of house dust as a specific excitant of asthma, there have been many attempts to characterize a hypothetical dust allergen. Its existence, as an entity, was recently questioned by Walzer[2] on the basis of critical examination of available clinical data pertaining to dust sensitiveness. He found among these data evidence of reactions which he attributed to non-specific irritative principles in extracts of house dust or other dusty substances. Noted also was the fact that no one had succeeded in sensitizing experimental animals to house dust[3] nor to suspected parent substances, cotton linters,[4] kapok fiber dust,[5] or mold[6] *(Aspergillus fumigatus)*.

Adaptation of the alum precipitation technic devised by Harrison[7] and employed by Caulfeild, Brown and Waters[8] in sensitizing guinea pigs to ragweed pollen extracts, has proved effective for establishing anaphylactic sensitiveness to water extracts of linters and house dust, respectively. Comparing anaphylactogenic properties of these extracts led to recognition of a non-specific factor in house dust extracts which induced anaphylactoid symptoms in unsensitized guinea pigs.

Two samples of clean, unused cotton linters were extracted with distilled water saturated with toluene. A 100 g sample was divided into 5 equal portions. To one 20 g portion sufficient solvent (100 ml) was added to saturate the fibers. After agitating for 48 hours, in a jar fixed on a rocking platform, the mixture was transferred to a Buchner funnel. The solution, recovered by suction and mechanical

1 Cooke, Robert A., *J. Immunol.*, 1922, **7**, 147.

2 Walzer, Matthew, *J. Allergy*, 1938, **10**, 72.

3 Ramsdell, Susan G., and Walzer, Matthew, *J. Immunol.*, 1927, **14**, 207.

4 Cohen, Milton B., Nelson, Tell, and Reinarz, B. H., *J. Allergy*, 1935, **6**, 517.

5 Wagner, H. C., and Rackemann, F. M., *J. Allergy*, 1936, **7**, 224.

6 van Leeuwen, W. S., Bien, Z., and Varekamp, H., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1923, **37**, 77.

7 Harrison, W. T., *U. S. Public Health Reports*, 1934, **49**, 462.

8 Caulfeild, A. H. W., Brown, M. H., and Waters, E. T., *J. Allergy*, 1936, **7**, 451.

pressure, was made up to 100 ml with water and used in the same manner to extract in succession the 4 remaining portions of the sample. Extract recovered from the last portion was made up to 100 ml and sterilized by Seitz filtration.

House dust samples from 2 sources* were heterogeneous mixtures of the sort obtainable from household vacuum cleaners. A 20 g sample of dust was mixed with 100 ml of distilled water saturated with toluene and was agitated on a rocking platform for 24 hours. After standing for 48 hours at 6°C, without agitation, the solid residues were separated and the sterile extract was recovered by Seitz filtration.

To the extracts obtained by the described procedures, a 10% solution of potassium alum was added to final concentration of 1% alum ($Al_2(SO_4)_3K_2SO_4.24H_2O$). Adjustment of reaction to pH 7 caused appearance of a copious precipitate. The mixture was allowed to stand 24 hours. For sensitizing, this neutral alum-treated extract was shaken and a dose of the suspension was administered subcutaneously. For control and shocking injections, the original sterile water extracts were used, with NaCl added to concentration of 0.9%, just before intravenous administration.

Anaphylactic sensitivity to the original water extracts of linters and dust, respectively, has been demonstrated in guinea pigs sensitized by subcutaneous injection of the alum-treated extracts. Guinea pigs were not sensitized after intraperitoneal injection of untreated water extract of linters, in 0.9% NaCl, administered in single or multiple doses totaling 5 or 6 ml.

Anaphylactoid symptoms, interpreted as evidence of a non-specific toxic factor, were observed in unsensitized animals following an initial intravenous injection of water extract of house dust. These symptoms were watering eyes, dyspnea and scratching of nose and eyes. Contractural spasms or coughing did not occur. Autopsy revealed emphysematous lungs, spotted with numerous punctate hemorrhages. Clotted blood was found in both thoracic and peritoneal cavities in animals dying from or sacrificed while recovering from anaphylactoid symptoms. Damage indicated by hemorrhagic areas was much more extensive than observed in animals exhibiting anaphylactic shock of like severity.

Recognition of an inherent toxic principle in house dust extract rests upon differences in doses required to induce ante- and post-mortem evidence of anaphylaxis in sensitized guinea pigs, and ana-

* Sample HD-E obtained from B. G. Efron, M.D., New Orleans, La. Sample HD-P obtained from H. S. Bernton, M.D., Washington, D. C.

TABLE I.

Animal No.	Body wt, g	Sensitizing dose	Sample	Incubation period, days	Body wt, g	Test injection, ml	Symptoms
78	330	1 ml Linters extr.	C-5	27	459	1.0	Moderate anaphylaxis
84	363	1 " " "	C-5	54	604	2.0	Severe "
142	355	2 " " "	C-5	133	739	2.0	Moderate "
444	318	2 " " "	C-16	29	520	3.0	" "
489	305	2 " " "	C-16	47	413	4.0	Fatal " 4 min.
435		None—Control	C-16		496	5.0	None
487		" "	C-5		418	4.5	"
490		" "	C-5		573	4.6	"
498		" "	C-16		665	5.0	"
479	237	2 ml Dust extr	HD-E	32	460	0.4	Fatal anaphylaxis 4 min
481	259	2 " " "	HD-E	39	390	0.4	" " 3 "
483	262	2 " " "	HD-E	57	598	0.4	Moderate "
486	247	2 " " "	HD-E	57	528	0.4	Fatal " 30 min.
566	276	2 " " "	HD-P	33	469	0.8	Fatal " 4 "
504		None—Control	HD-E		473	1.0	None
509		" "	HD-E		340	2.0	Moderate anaphylactoid
510		" "	HD-E		327	2.0	" "
503		" "	HD-E		416	2.0	Fatal "
564		" "	HD-P		465	0.8	None
567		" "	HD-P		470	0.8	"
573		" "	HD-P		331	3.0	Moderate anaphylactoid

phylactoid symptoms in the unsensitized animals. Whether the inherent toxic principle detected in two samples of house dust is responsible for non-specific positive cutaneous reactions in human subjects has not been determined. However, in associated clinical trials, house dust extract "E" has been found to possess skin whealing agents of unusual potency.

Specimen results appear in Table I.

10456

Aortic Pressure and the Diastolic Volume Law of Energy Output in Cardiac Contraction.

GORDON K. MOE, EARL H. WOOD AND MAURICE B. VISSCHER.

From the Department of Physiology, University of Minnesota, Minneapolis.

The conclusion of Starling and Visscher[1] that the oxygen consumption of the isolated heart under constant chemical and temperature conditions is determined solely by the initial length of its muscular fibers has been the subject of some disagreement. Stella[2] asserted that at constant initial fiber length, the energy output of the tortoise ventricle in contraction varied with the arterial resistance. It was shown, however, by Moldavsky and Visscher[3] that a systematic error in measurement of diastolic ventricular volume occurred in Stella's experimental procedure and that when this error was obviated by suitable means the energy output in contraction was found to depend only upon initial fiber length and was entirely unrelated to pressure or work conditions.

Kiese and Garan[4] have attacked this problem again with the mammalian heart-lung preparation. In a series of exact studies they have shown that the oxygen consumption of the heart is significantly greater when a diastolic ventricular volume increase is brought about by raising the inflow, and therefore the minute volume, at constant arterial pressure, than when the same diastolic volume is obtained by increasing the aortic pressure, keeping the inflow constant at a low level. Stated in numerical terms, they found in a typical experiment that in order to have identical oxygen consumptions the

1 Starling, E. H., and Visscher, M. B., *J. Physiol.*, 1927, **62**, 243.

2 Stella, G., *Ibid.*, 1931, **72**, 247.

3 Moldavsky, L. F., and Visscher, M. B., *J. Physiol.*, 1937, **91**, 23.

4 Kiese, M., and Garan, R. S., *Arch. f. Exp. Path. u. Pharmak.*, 1938, **188**, 226.

diastolic volume had to be about 5 cc greater when the arterial pressure was 200 mm of mercury, than when it was 100.

Kiese and Garan measured the ventricular volume with a Henderson cardiometer and did not take into account the obvious fact that the coronary vessels themselves, particularly the larger arteries on the surface of the heart, are elastic chambers and that the volume of blood within them depends upon the aortic pressure. Changes in the volume of blood in the coronary vessels influence the apparent volume of the ventricles but do not correspondingly change the length of the heart muscle fibers. In order to determine what rôle, if any, this factor plays in measurements of the external ventricular volume, it was necessary to have a quantitative measure of the volume of blood within these vessels at various perfusion pressures while the heart was beating. We have, therefore, made several hundred measurements on 6 separate hearts in which in effect the blood in the coronary system has been weighed. A cannula carried blood from a pressure reservoir to the brachiocephalic artery in which it was inserted. A length of 20 cm of thick-walled rubber tubing connected this cannula with a warming coil which was rigidly fixed, and through which the blood passed from the reservoir to the coronary system. The aorta was tied distal to the cannulated vessel. The heart was suspended by a rigid hook inserted in the musculature of the apex which was attached to the arm of a spring steel torsion lever. An extension of this lever, recording on a smoked drum, was used to record the changes in position of the torsion lever. By means of this device, the weight of the heart was continuously recorded. To permit free drainage of the coronary blood, the atrioventricular valves were cut and the venous blood was collected in a large funnel under the heart. The blood from the heart passed through an oxygenator and was pumped back mechanically to the reservoir. Hearts set up in this manner will beat spontaneously for periods up to 5 hours. In some experiments the heart rate was controlled by an artificial stimulating device. With each contraction of the heart, there is a swing of the lever which is sufficiently damped to come to rest during the diastolic pause. The torsion lever was calibrated by weights hung on the beating heart. The changes observed with alterations in perfusion pressure were found to be perfectly reversible. The sensitivity of the whole system was usually such that 10 g of added weight produced a lever deflection of 12 mm.

Fig. 1 presents the results of a typical experiment in which variations in perfusion pressure were made. It will be noted that the weight of the whole heart increases steadily as the perfusion pressure is raised. The effect is completely reversible, and in view of the con-

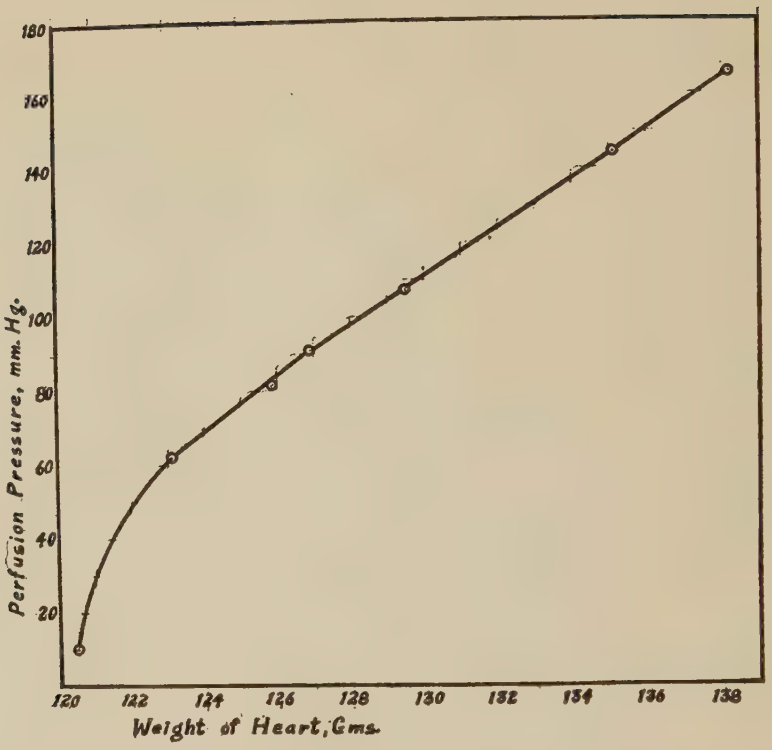


FIG. 1.

Effect of perfusion pressure upon the volume of blood contained in the coronary vessels.

ditions of the experiment it can be concluded that the change in weight is probably an exact measure of the change in the volume of blood within the coronary vessels under the several perfusion pressures. Comparable results were obtained in each one of the experiments.

In order to substantiate the conclusion that the weight change measurements are actually a reflection of volume changes within the coronary vessels, a known coronary constrictor drug was introduced. 1/10 cc of Parke-Davis Pitressin was introduced into the blood flowing into the coronaries which produced a decrease in weight of 4.7 g.

Further studies were made of the effect of rate changes, ventricular fibrillation and adrenaline injections. It was found that the coronary volume was less at higher heart rates and that it was considerably greater in the fibrillating heart. During the latter state, adrenaline still further increased the capacity of the coronary vessels.

It is of interest to employ these data in the interpretation of the findings of Kiese and Garan. The latter workers noted an unex-

pectedly small oxygen consumption at the higher aortic pressures. In the light of the present results, it will be obvious that this is exactly what might be predicted from the fact that the measured external diastolic volume is not a true measure of muscle fiber length when the coronary perfusion pressure is altered. The large coronary vessels on the surface of the heart stretch under the influence of internal pressure without any concomitant increase in fiber length. Likewise, in the case of arterioles, capillaries and veins, only those nearest the endocardium could cause muscle fiber extension comparable to their volume increase. As noted above, for a difference in perfusion pressure of 100 mm of mercury, Kiese and Garan found a disparity of about 3 to 5 cc in the diastolic ventricular volumes necessary to produce equal oxygen consumptions. Reducing our average results to hearts of the size used by the previous workers, we find that a rise in perfusion pressure of 100 mm of mercury increases the volume of blood within the coronary vessels by 8 cc. Since some of this increase in weight is due to blood within the walls of the heart, in which situation it would result in some increased fiber length, we interpret our observations to indicate that the discrepancies found by Kiese and Garan can be completely accounted for on the basis of the error introduced in external diastolic volume measurements by neglecting the volume of blood in the coronary vessels having little or no influence upon fiber length.

Conclusions. When very small changes in diastolic ventricular volume are measured one cannot disregard the coronary perfusion pressure as a factor in determining the volume of blood in the coronary vessels. Since the volume of blood in the superficial vessels has no influence upon the fiber length, and the quantity in the deeper vessels has a lesser influence than does the volume within the ventricles, it is obvious that corrections in the measured external diastolic ventricular volume must be made in order to compare energetic conditions at various perfusion pressures. The quantitative results obtained here are of the right order of magnitude to account completely for the small discrepancies found by Kiese and Garan in energy liberation at various aortic pressures. It is believed that the present results invalidate the conclusion that the aortic pressure is a determining factor in the energy liberation by heart muscle. The best evidence therefore indicates that the conditions of loading, other than those determining the initial fiber length, or diastolic ventricular volume, have no influence upon the energy liberated in cardiac contraction. This conclusion is most fully substantiated by the fact that in the tortoise ventricle, where there is no coronary system, the diastolic volume law obtains irrespective of the type of loading.

10457 P

Rôle of Respiratory Obstruction in "Neuropathic" Pulmonary Edema Following Vagotomy.

VICTOR LORBER. (Introduced by M. B. Visscher.)

From the Department of Physiology, University of Minnesota.

Reports by Weiser[1] and Farber[2] have suggested that pulmonary congestion and edema, fatal within a period of hours, are specific consequences of bilateral vagotomy in the rat, guinea pig, and rabbit. In the larger common laboratory animals (cat and dog) this is not the case.[3, 4, 5] This study was undertaken in an effort to determine the basis for this difference.

Doubly vagotomized rats and guinea pigs in which tracheotomy was first done were kept alive many hours longer than the longest survivals reported,[1, 2] without evidence of pulmonary edema. Tracheotomy consisted of a simple transverse slit, handling of the trachea being avoided. The animals were fixed on their backs throughout the procedure to permit care of the wound. Cannulation, which would have circumvented this, was found unsatisfactory because of the difficulty in maintaining a free air-way. In spite of all precautions, tracheal secretions produced respiratory obstruction in a number of animals. Death with pulmonary congestion and edema followed shortly if effective steps were not taken to clear the air-way.

In a number of rats and guinea pigs, and in one rabbit, the right vagus was cut below the recurrent laryngeal nerve, the left being severed in the neck a number of days later. In the rat, paralysis of one vocal cord caused an obvious obstruction, the animal dying within thirty hours, many times the survival period observed in bilaterally vagotomized rats with both vocal cords immobilized. The lungs exhibited varying degrees of congestion and edema, as well as extensive pneumonic consolidation. Survival in the guinea pigs ranged from one to 22 days. Laryngeal obstruction was apparent in the short survivals. The lungs were similar to those in the rat. Obstructive symptoms were minimal in the longer survivals. These were terminated by pneumonia. The rabbit lived 10 days, the lungs at death showing pneumonic consolidation.

---

1 Weiser, J., *Pflüger's Arch.*, 1932, **231**, 68.

2 Farber, S., *J. Exp. Med.*, 1937, **66**, 397, 405.

3 Schafer, E. S., *Quart. J. Exp. Physiol.*, 1919-20, **12**, 231, 367.

4 Boothby, W. M., and Shamoff, V. N., *Am. J. Physiol.*, 1915, **37**, 418.

5 Pavlov, I. P., *The Work of the Digestive Glands*, 2nd Ed., London, 1910.

It was concluded that respiratory obstruction was primarily responsible for the lung changes observed, and that the reaction of smaller animals to bilateral vagotomy, in this particular respect, differed in no way from that of the larger animals, but that it was less readily demonstrated because of a smaller airway which easily became occluded.

10458

In vitro Studies on the Action of Sulfapyridine.

ROBERT E. HOYT AND MILTON LEVINE. (Introduced by R. N. Bieter.)

From the Departments of Bacteriology and Pharmacology, University of Minnesota.

The use of sulfapyridine (2-sulfanilyl aminopyridine) in experimental pneumococcus pneumonia,[1] together with favorable clinical reports in the treatment of lobar pneumonia, led Fleming[2] to conduct *in vitro* studies on the action of the drug on the pneumococcus. He observed a marked bacteriostatic effect in deleukocyted blood, using his "slide-cell" technic. His criterion of bacteriostasis was the relative colony size of drug-treated cultures as compared with untreated controls. Whitby[1] reports a degeneration and final disappearance of the capsule in the peritoneum after 4 hours' growth in drug-treated mice. Fleming[3] has not been able to confirm this in his *in vitro* work.

The present studies were made to obtain data regarding the growth curve of the pneumococcus under the influence of the drug, which would give a quantitative measurement of bacteriostasis. In addition, morphological studies were made at regular intervals during the growth curve.

The organism used was a Type II pneumococcus which had been carried in mice for over a year by the method of Neufeld and Handel.[4] Eight-hour cultures, made by adding a drop of heart's blood from a freshly dead mouse to 20 cc of veal infusion broth, were used in all experiments. A culture of this age was chosen to prevent the appearance of a lag phase, which would result in exposure of resting

1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Fleming, A., *Lancet*, 1938, **2**, 74.

3 Fleming, A., *Lancet*, 1938, **2**, 564.

4 Neufeld, F., and Handel, L., *Berl. Klin. Woch.*, 1912, **49**, 680.

cells to prolonged action of the drug. This is in accordance with previous work by Chesney,[5] showing that subcultures made during or immediately following the logarithmic growth phase exhibit little or no lag.

Two cubic centimeters of a 1:1000 solution of sulfapyridine were added to 18 cc of the menstruum to be tested. This made a final concentration of 1:10,000 of the drug. Two cubic centimeters of saline were added to the controls. Each tube received ½ cc inoculum of the whole pneumococcus culture.

Each hour 1 cc samples of the cultures being examined were diluted, pipettes being changed with each dilution. Plates were made with 3% rabbit-blood agar. Each calculated point on the curve represents the average of 10 plates.

The growth studies presented are representative ones chosen from the experiments. Fig. 1 shows curves obtained by growing the organism in 2% peptone (Parke-Davis) veal infusion broth, with and without the drug. No significant difference is to be observed due to the presence of the drug.

Lockwood[6] has shown that the action of sulfanilamide upon streptococci is inhibited in the presence of peptone. Fig. 2 shows the curves obtained by growing the organisms in peptone-free veal infusion broth. A significant bacteriostatic action by the drug is seen; the number of organisms in the treated culture rises more slowly, and at 8 hours, is approximately half that of the control.

Measurements were made of the number of organisms growing in uninactivated rabbit serum. Samples were sealed and rotated according to the method of Todd.[7] Fig. 3 shows curves obtained under these conditions. Marked bacteriostasis is observed, much greater than in the peptone-free broth, although the serum control rises to a higher point than the veal-infusion control.

When peptone sufficient to make a final concentration of 2% was added to serum, the effect of the drug was largely lost, as is shown in Fig. 4.

It has been shown that in the absence of peptone, sulfapyridine in a concentration of 1:10,000 exerts a pronounced bacteriostatic effect upon growing pneumococci. This is especially apparent in the whole uninactivated serum.

Slides made at hourly intervals were stained by Maneval's[8] method

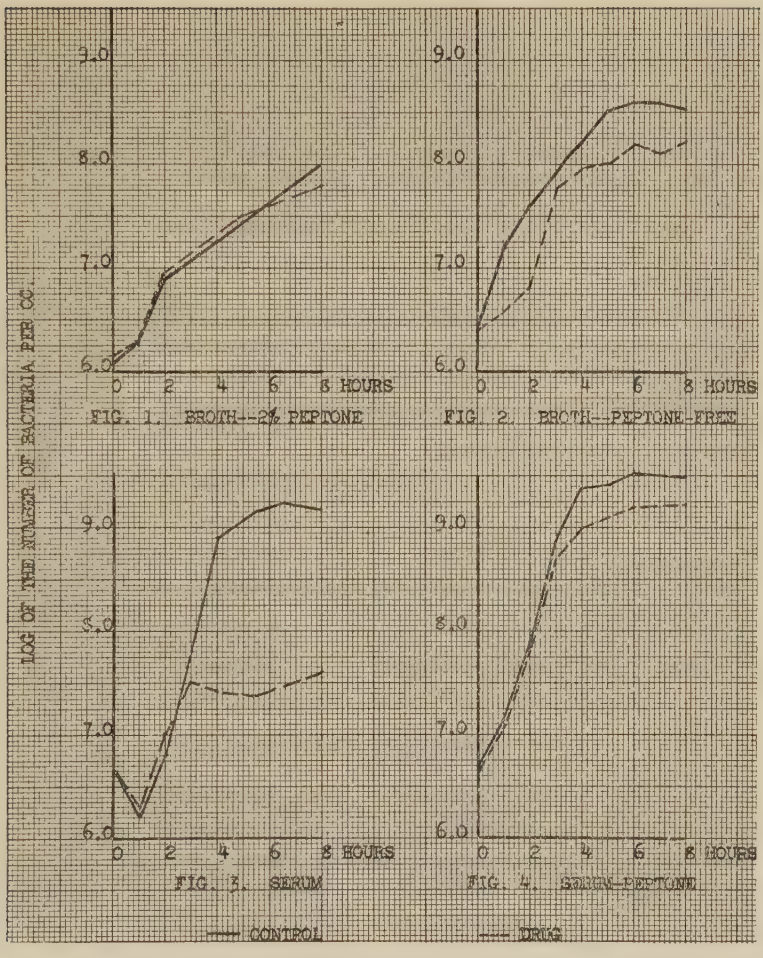
---

5 Chesney, A. M., *J. Exp. Med.*, 1916, **24**, 387.

6 Lockwood, J. S., *J. Immun.*, 1938, **35**, 155.

7 Todd, E. W., *Brit. J. Exp. Path.*, 1927, **8**, 1.

8 *Stain Technology*, 1928, **4**, 21.



for the demonstration of capsules. No inhibition of capsule formation could be seen in the drug-treated cultures. In peptone-free serum cultures, the organisms growing in the presence of the drug showed a marked tendency to grow in long chains of 10-18 cocci, whereas in the controls, the diplococcus form was predominant, with some short chains of 4 or 6 cocci.

We are indebted to Merck and Co., Inc., for the supply of sulfapyridine.

10459 P

Effect of Adrenalectomy upon Intestinal Absorption of Sodium Chloride.

WILLIAM G. CLARK. (Introduced by Maurice B. Visscher.)

From the Department of Zoology, University of Minnesota, Minneapolis, and the Scripps Metabolic Clinic, La Jolla, Calif.

It is well established that adrenal insufficiency provokes a negative salt and water balance in most mammalian organisms which have been studied, and that this condition can be corrected for the most part by cortical hormone and/or salt therapy. Several workers have reported that this negative balance is due to a primary renal effect; some have indicated it to be due also to primary extra-renal disturbances; and still others have stated that the electrolyte disturbances are secondary to disturbances in carbohydrate metabolism.

In another study[1] it was found that healthy adrenalectomized rats, maintained on Rubin-Krick salt drinking-fluid and stock diet for periods up to 2 weeks, absorbed glucose at the same rate as controls. A modified Cori technic was used, employing the intact rat. This finding confirmed Deuel, *et al.*,[2] who also used this technic; and failed to confirm the conclusions of Wilbrandt and Lengyel,[3] and Verzar, *et al.*,[4] who used the isolated intestinal loops of rats, cats, and dogs.

The present study indicates that healthy adrenalectomized rats absorbed NaCl more slowly than controls. The Cori technic was used in preliminary studies, and both sodium[5, 6] and chloride[7, 8] of the minced, leached gastrointestinal tract was determined in most cases, although this was not essential since stomach-emptying of control and adrenalectomized rats was comparable for both sodium and chloride. The stomach-emptying of the adrenalectomized rats varied far more than that of the controls, however, so that for small differences in intestinal absorption, the stomachs should be separately analyzed.

The intestinal absorption rates of both Na and Cl were comparable

1 Clark, W. G., and MacKay, E. M., in preparation.

2 Deuel, H. J., Jr., Hallman, L. F., Murray, S., and Samuels, L. T., *J. Biol. Chem.*, 1937, **119**, 607.

3 Wilbrandt, W., u. Lengyel, L., *Biochem. Z.*, 1933, **267**, 204.

4 Issekutz, B. V., Jr., Laszt, L., u. Verzar, F., *Arch. ges. Physiol.*, 1938, **240**, 612. (*Cf.* for earlier papers.)

5 Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

6 Hoffman, W. S., and Osgood, B., *J. Biol. Chem.*, 1938, **124**, 347.

7 Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1923, **58**, 523.

8 Sendroy, J., Jr., *J. Biol. Chem.*, 1937, **120**, 405.

and those of the adrenalectomized animals were considerably less than the controls, although more variable.

No differences were found between adrenalectomized and control animals with respect to: concentrations of Na and Cl of the tissues of stomachs and intestinal tracts, concentrations of Na and Cl of the serum, hemoglobin concentrations of the blood, body weight changes, body (rectal) temperatures, and general activity. That the rats lacked cortical hormone was proved by the fact that they died of adrenal insufficiency within a few days after removal of the salt drinking fluid, and that they exhibited a marked diuresis. No effect of cortical hormone injections was found on intestinal absorption of NaCl by normal sham-operated controls. These details will be published elsewhere.

Later, a method of determining intestinal absorption similar to that developed by Barnes, *et al.*,[9] was used, separately rinsing residual, unabsorbed NaCl from the stomach and intestine of the subsequently etherized rat with isotonic, warmed sucrose after a definite absorption period following administration of NaCl to the intact animal (1 cc 3% NaCl per sq dec body surface). The results are summarized in Fig. 1 and are expressed as residual Cl found in the intestine as percent of the amount of Cl available for absorption by the intestine, after correcting for Cl found in the stomach. Thus the amount available would be the difference between the amount fed and the amount found in the stomach. The rinse method, however, requires no correction for chlorides which are leached from the tissues of the gastrointestinal tract when the Cori method is used.

In the author's opinion, this method is superior to the isolated loop method, since trauma associated with an abdominal incision and intestinal manipulation causes severe circulatory disturbances and shock in the adrenalectomized animal[10, 11] and would thus vitiate results obtained on the experimental animals. Experiments have shown that the NaCl obtained by rinsing the alimentary tract with isotonic sucrose 2-3 minutes after administration, represents approximately 96% of the amount fed. The 4% loss is understandable when it is considered that 1 drop of the 3% solution contained approximately 3 mg NaCl, and a drop or two in the oesophagus and mouth would decrease the recovery several percent. Furthermore,

[9] Barnes, R. H., Wick, A. N., Miller, E. S., and MacKay, E. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, —

[10] Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 677.

[11] Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 22. (*Cf.* for earlier papers.)

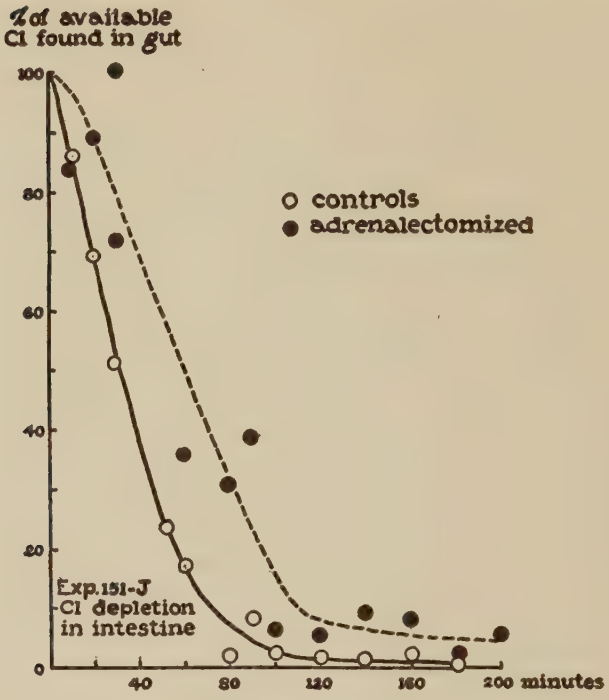


FIG. 1.

Hamilton[12] showed that radioactive salts may appear in the extremities 3-5 minutes after oral administration of isotonic solutions to the human.

Fig. 1 shows the decreased rate of intestinal absorption of Cl in the healthy, active adrenalectomized rat. As pointed out above, other experiments have shown that Na behaves just as Cl. Practically 100% absorption has occurred in approximately 90 minutes.

The results of this work, experimental details of which will appear elsewhere, and those of the previous work on glucose absorption, support the theory that the electrolyte metabolism of the adrenalectomized animal is affected by a primary disturbance of membrane behavior rather than by a secondary disturbance in the phosphorylation of glucose. The results also indicate that this primary disturbance may occur in membranes other than in the kidney. Further aspects of this membrane disturbance are being investigated at the present time.

[12] Hamilton, J. G., *Am. J. Physiol.*, 1938, **124**, 667.

10460

Relation of Acetyl Choline to Anaphylactic Shock in the Rabbit.*

OSCAR D. RATNOFF. (Introduced by B. C. Seegal.)

From the Departments of Bacteriology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York.

The release of a histamine-like substance into the blood stream during anaphylactic shock, as first suggested by Dale and Laidlaw,[1] has been fairly well established. Dragstedt has isolated a histamine-like substance from the blood stream and thoracic duct of intact animals during anaphylaxis;[2] and this substance has been demonstrated in the perfusate of an isolated guinea pig lung during anaphylactic shock.[3, 4]

Discrepancies between the syndrome of anaphylaxis and the pharmacological action of histamine have led to the search for other "humors" to explain the former phenomenon. Schittenhelm and his coworkers showed that the serum potassium rose markedly in rabbits and dogs during anaphylactic shock.[5] Went and Lissák claim to have isolated choline from the perfusate of an isolated guinea pig heart during anaphylactic shock.[6]

Wenner and Buhrmester report that acetyl choline can be detected in rabbit's heart blood during anaphylaxis.[7] This liberation of acetyl choline in the blood was measured both by Fühner's sensitized leech preparation,[8] and by the effect on a cat's blood pressure of an extract made according to Bischoff and Kapfhammer's method.[9] Although the leech technic is more sensitive, Wenner and Buhrmester noted that in some experiments, with no measurable concen-

* I would like to thank Drs. B. C. Seegal and E. G. Miller, Jr., for their kind advice and encouragement.

1 Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1910, **41**, 318.

2 Dragstedt, C. A., and Gebauer-Fuelnegg, E., *Am. J. Physiol.*, 1932, **102**, 512, 520.

3 Bartosch, R., Feldberg, W., and Nagel, E., *Pflüger's Arch.*, 1932, **230**, 129, 674; 1933, **231**, 616.

4 Spinelli, A., *Boll. Sez. ital. Soc. int. Microbiol.*, 1932, **4**, 257.

5 Schittenhelm, A., Erhardt, W., and Warnat, K., *Klin. Wochenschr.*, 1927, **6**, 2,000.

6 Went, S., and Lissák, K., *Arch. Exp. Path. u. Pharm.*, 1936, **182**, 509.

7 Wenner, W., and Buhrmester, C., *J. Allergy*, 1937, **9**, 85.

8 Fühner, H., *Arch. Exp. Path. u. Pharm.*, 1917, **82**, 81; *Biochem. Z.*, 1918, **92**, 347.

9 Bischoff, C., and Kapfhammer, J., *Z. Physiol. Chemie*, 1930, **191**, 179.

tration of acetyl choline could be detected by this means, Bischoff and Kapfhammer extracts would show "considerable amounts of acetyl choline." Concentrations of 1:1,000,000 to 1:10,000,000 were reported for the final concentrated extract of heart's blood of rabbits dying in shock.[10]

Although normal rabbit's blood will not cause contraction of the sensitized leech, by the Bischoff and Kapfhammer method it will show an acetyl choline concentration of about 0.05 gamma per cc, or 1:20,000,000.[11] Since Wenner and Buhrmester's figures are for a concentrated extract, and not for whole blood, it would seem that the concentration of acetyl choline found by them was within normal limits for this method. It is to be noted that these workers made no attempt to inhibit the very rapid *in vivo* destruction of acetyl choline by the blood esterase.

Experimental. Detection of Acetyl Choline in the Blood of the Shocked Animal: A group of rabbits was sensitized to whole egg albumen according to Grove's method.[12] The animals were injected with 1 cc of 50% egg albumen intravenously, followed 5 and 10 days later by 5 cc of egg albumen intraperitoneally; and then by 7 daily intraperitoneal injections of 1 cc each. Seven days after the last injection the rabbits were given 1.5 cc of antigen subcutaneously and were finally shocked on the 31st day with 3 to 5 cc of 50% egg albumen given intravenously.

Of 16 rabbits, 11 survived the course of sensitization, and were shocked a total of 12 times. Three animals were shocked without previous physostigmine injection; these animals showed 2 severe, and 1 mild shock. In 9 rabbits the injection of egg albumen was preceded by 0.15 mg/kilo of physostigmine intravenously. The physostigmine was administered to prevent the otherwise rapid destruction of acetyl choline by the blood choline esterase. Each of these 9 was shocked severely enough to die following the added insult of the withdrawal of heart's blood. At the first symptoms of shock, heart's blood was withdrawn into a syringe containing a few drops of 8% solamine fast pink, an analogue of the anticoagulant dye chlorazol fast pink, to which had been added physostigmine solution, 1:100,000. The blood was then added to a leech muscle preparation sensitized at room temperature with 1:100,000 physostigmine in frog Ringer's solution. Viability of the leech was always tested with known concentrations of acetyl choline; the effectiveness

[10] Personal communication.

[11] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[12] Grove, Ella, *J. Immunology*, 1932, **23**, 101.

of the physostigmine in inhibiting the choline-esterase was tested by adding known amounts of acetyl choline to the eserinized heart's blood. In 11 of 12 experiments the leech was sensitive to at least 1×10^{-7} of acetyl choline; usually about 2 or 3×10^{-8} caused a vigorous contraction.

In no case did the leech muscle contract following the addition of the shocked rabbit's blood. On the contrary, relaxation of the leech occurred in some instances.

Summary. No direct evidence could be found to support the view that acetyl choline is released into the blood stream in detectable amounts in anaphylactic shock in the rabbit.

10461 P

Transmission of Endocarditis Lenta to Rabbits.

WARD J. MACNEAL, MARTHA JANE SPENCE AND MARIE WASSEEN.

From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

Rosenbach[1] produced experimental bacterial endocarditis in dogs by passing an instrument down the right carotid artery to wound the valves. Most of his experiments on dogs and rabbits produced aseptic lesions but 2 of his dogs developed large vegetations on the aortic valve and numerous septic infarcts and petechial hemorrhages in various organs. Colonies of micrococci, apparently staphylococci, were demonstrated in these lesions. Ever since that pioneer observation there has prevailed the idea that some accessory injury of the endocardium is necessary in addition to the introduction of bacteria into the blood-stream, if one is to succeed in causing experimental endocarditis. Welch, Murdock and Ferguson[2] punctured the heart and then rotated the needle to produce a preliminary endocardial wound before introducing their bacteria intravenously or into the pulp of a tooth. In addition they resorted to accessory respiratory infection and dietary restriction to lower the resistance of their animals. In this way they succeeded in producing gross endocardial vegetations in 20% of their rabbits inoculated with endocarditis-

1 Rosenbach, Ottomar, *Arch. f. exp. Path. und Pharm.*, 1878, **9**, 1.

2 Welch, Henry, Murdock, Thomas P., and Ferguson, John A., *J. Lab. and Clin. Med.*, 1936, **21**, 1264.

streptococci. Dietrich[3] has introduced various substances parenterally to injure the valves and then has injected staphylococci or colon bacilli to produce the vegetations. British investigators, however, have been successful without the preliminary injury to the endocardium. Apparently the first success of this kind was obtained by Dreschfeld,[4] who succeeded by the simple intravenous injection of endocarditis-streptococci into rabbits long before *Streptococcus viridans* had been distinguished from other streptococci. More recently Lloyd-Jones[5] has made a most significant contribution. He was able to produce endocarditis in rabbits with great regularity by repeated daily intravenous injection of hemolytic streptococci and particularly by such injection of serum-broth cultures of viridans types of streptococci obtained from the human intestine and uterine cervix.

For our more successful experiments we have used serum-broth cultures of various strains of *Streptococcus viridans* isolated from the blood of clinical cases of endocarditis lenta. The culture has been injected into the ear vein of a rabbit in doses of 1 to 4 cc daily for six days with a pause for 48 hours. Then a blood culture has been taken and the series of six daily injections repeated. This program has been continued until two or three successive positive blood cultures have been obtained and the animal has shown a definite loss of weight. Then the inoculations have been discontinued and the disease allowed to take its course or, in some instances, various therapeutic procedures have been undertaken, which cannot now be discussed. Weekly or more frequent blood cultures were continued and the body weight has been observed. Careful necropsy has been performed as soon as possible on all the animals that have died. Up to February 12, 1939, the series includes 21 rabbits already dead. Of these there were 12 with definite vegetative endocarditis and a glance at Table I will show that the positive results have been obtained with considerable regularity in the recent series. It should be noted, however, that positive results have been obtained so far with only two bacterial strains and standard results are being obtained with only one. We are undertaking experiments with other bacterial strains at the present time.

The lesions recorded as positive have been large and easily recognizable in the gross examination. In sections of the vegetations un-

[3] Dietrich, Wolfgang, *Virchow's Arch.*, 1937, **299**, 285.

[4] Dreschfeld, *Brit. Med. J.*, 1887, **2**, 887.

[5] Lloyd-Jones, D. M., An experimental study of malignant endocarditis, Appendix to *Bacterial Endocarditis*, pp. 113-137, John Wright and Sons, Ltd., Bristol (Eng.), 1936.

TABLE I.
Rabbits Inoculated Intravenously with *Streptococcus viridans.*

Rabbit	Strain	Initial Inoculation	Death	Significant lesions
12	M	Jan. 19, 37	Mar. 4, 37	Mitral vegetation
11	S	" 19	" 12	Negative
19	M	" 30	Apr. 12	"
466	W	Feb. 8	Feb. 11	"
467	G	" 8	" 15	"
458	P	Dec. 5, 38	Dec. 11, 38	Uncertain
143	P	" 5	" 16	Negative
148	P	" 5	" 19	" (?)
345	P	" 5	" 20	" (?)
469	P	" 5	" 23	Aortic vegetation, splenic infarct
472	P	" 5	" 27	Negative
304	P	" 5	" 30	Mitral vegetations
302	P	" 5	" 30	Tricuspid vegetations, renal infarct
465	P	" 5	Jan. 5, 39	Mitral vegetation
316	P	" 28	" 11	Mitral and mural vegetations, kidney, lung
315	P	" 28	" 16	Mitral vegetation
303	P	" 28	" 25	" "
317	P	" 28	" 28	" "
313	P	" 28	Feb. 1	Tricuspid, aortic, mitral vegetations
307	P	" 28	" 4	Tricuspid, mitral vegetations
309	P	" 28	" 12	Mitral vegetation, lung

der the microscope one can easily recognize the colonies of streptococci in the necrotic substance and the inflammatory reaction in the adjacent living fibroblastic granulation-tissue of the lesions.

We conclude, therefore, that it is possible to transmit endocarditis lenta from man to the rabbit by repeated intravenous injection of pure cultures of the specific bacteria, without accessory injury or other depression of the animal. It is hoped that there may thus be provided suitable material for detailed study of the experimental disease and for the testing of various therapeutic measures.

10462 P

Qualitative Separation of Proteins of Rat Serum by Electrophoresis.

E. Jameson and C. Alvarez-Tostado.

From the Departments of Medicine and Chemistry, Stanford University.

The electrophoretic migration of the proteins in rat serum was studied in the apparatus modified by Tiselius from older designs.[1] Toeppler's "Schlieren" method was used for following the movement of the differently charged fractions.

Three different fractions, one albumin and 2 globulins, separate from normal rat serum under the influence of a low difference of potential (about 135 volts, giving about 26 milliamperes current). Electrophoresis was continued as long as 11 hours without the occurrence of any further split in the protein bands. Human and horse sera,[2] studied in this and other laboratories, differ from rat serum in that they contain an albumin and 3 globulin fractions. (Fig. 1.)

Rat serum continued to yield the same number of fractions at different pH values extending on both sides of the isoelectric points of the proteins from pH 4.0 to 7.5. (Fig. 2.) An exception occurs when a globulin is partially precipitated at a pH near its isoelectric point. In this case a fourth fraction appears.

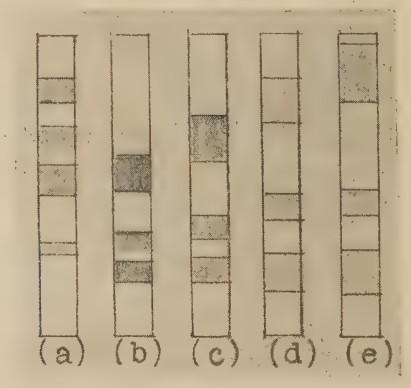


Fig. 1.
Electrophoresis of horse serum (a), and rat serum at pH 6.23 3, 4, 5, and 6 hours after potential was applied (b, c, d, and e).*

1 Tiselius, A., *Tr. Farad Soc.*, 1937, **33**, 524.

2 Tiselius, A., *Särtryck ur Svensk Kemisk Tidskrift*, 1938, **50**, 66.

* Buffer solutions are .02 molar with respect to the phosphate ovine acetate ion and .15 molar with respect to NaCl.

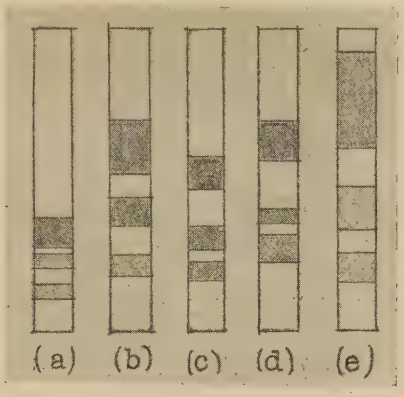


FIG. 2.
Electrophoresis of rat serum at pH values of 4.0 (a), 4.95 (b), 6.23 (c), 7.0 (d), and 7.5 (e).*

If electrophoresis is carried out at the low potential until the 3 fractions have been well separated and the potential is then raised to 250 or 300 volts (50 to 60 milliamperes current) a disintegration of the 2 globulins takes place. At pH 7.5, the α globulin gives rise to a fraction travelling with greater speed and appearing, consequently, between the albumin and the α globulin. The β globulin yields a fraction moving with less speed, and visible as a rather sharp line following this fraction. At a pH value below the isoelectric point of the globulins, 4.95, the fraction separating from the β globulin precedes it towards the negative electrode; the fraction with its source in the α globulin precedes the albumin towards the positive electrode. These new fractions persist after the voltage is returned to its original value. (Fig. 3.)

FIG. 3.
Electrophoresis of rat serum, showing serum at pH 7.5 (a) 135 volts, (b) 270. At pH 4.95 (c) 135 volts, (d) 270 volts.*

These experiments appear to confirm the view, now held by several investigators besides ourselves,[3] that globulins may occur in complexes, easily resolved into their components. In this instance the resolution has been effected by applying a sufficient potential gradient.

10463

Assay of Vitamin K Concentrates.*

SIDNEY A. THAYER, R. W. MCKEE, S. B. BINKLEY, D. W. MACCORQUODALE AND EDWARD A. DOISY.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Mo.

Since our extensive experience in the assay of estrogens has led to an appreciation of the importance of the contribution of Trevan[1] to bioassay, our work on the assay of Vitamin K was directed toward the establishment of standard curves of response to the administration of the vitamin. Based on Trevan's principles a curative method of assay which appears to have certain advantages over methods previously used by Schønheyder,[2] Almquist and Stokstad,[3] and Almquist, Mecchi and Klose[4] has been developed.

Experimental Diet. In our earlier work, difficulty in consistently producing the deficiency was encountered. It seems that this was due chiefly to diets that were not entirely devoid of Vitamin K. The diet now used is one described by Almquist.[5] Its composition is: fish meal, 17.5 parts; dried brewer's yeast, 7.5 parts; ground polished rice, 73 parts; sodium chloride plus small amounts of cupric and ferrous sulfates, 1.0 part; and cod liver oil 1.0 part. The fish meal and yeast were extracted with hot isopropyl ether before incorporation in the diet.

---

3 Jameson, E., *Symposia on Quantitative Biology*, Cold Spring Harbor Biological Lab., 1938, **6**, 331; Sørensen, S. P. L., *Kolloid Z.*, 1930, **53**, 102; Kendall, F. E., *J. Clinical Invest.*, 1937, **16**, 921; McFarlane, A. S., *Biochemical J.*, 1935, **29**, 660, etc.; and others.

* We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

1 Trevan, J. W., *Proc. Roy. Soc.*, 1927, **101**, 483.

2 Schønheyder, F., *Biochem. J.*, 1936, **30**, 890.

3 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.

4 Almquist, H. J., Mecchi, E., and Klose, A. A., *Biochem. J.*, 1938, **32**, 1897.

5 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

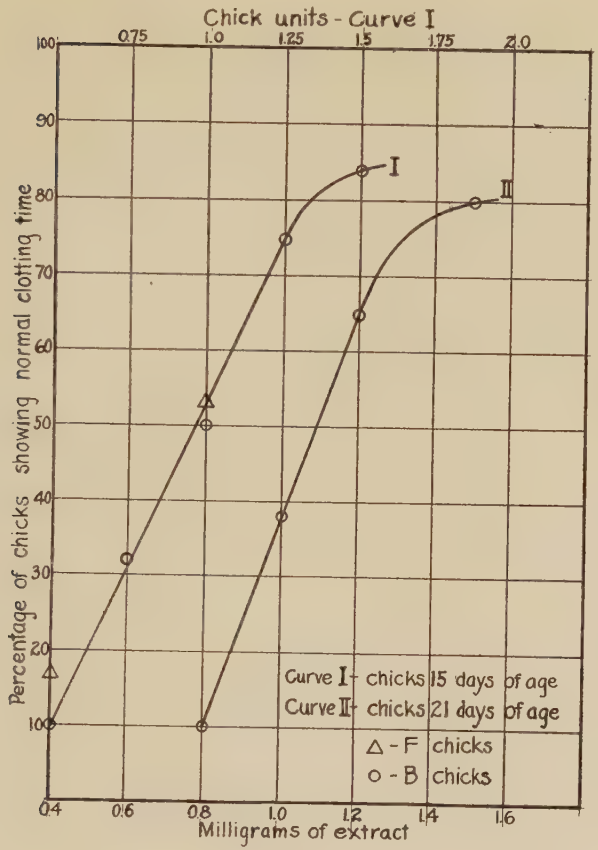


FIG. 1.

Using this diet it has been found that a severe deficiency can be produced within 2 weeks even in AAA grade chicks (B) obtained from a commercial hatchery. Chicks (F) from eggs obtained from hens which had been on a diet devoid of greens seemed to develop only a slightly greater degree of deficiency. (Fig. 1.)

Assay. The vitamin dissolved in 0.6 cc of sesame oil was given orally, 0.20 cc of solution being administered on each of 3 successive days.† The solution was administered from a tuberculin syringe through a tube (1x90 mm) which was inserted directly into the crop. On the fourth day approximately 0.5 cc of blood was drawn from the brachial vein into a small porcelain crucible, and the time for coagula-

† Perhaps the single administration used by Ansbacher (*Science*, 1938, **88**, 221) may prove to be more satisfactory than the divided administration employed in our work. Certainly, the shorter time required for assay is a point in its favor.

tion at room temperature determined. A clotting time of 10 minutes or less was arbitrarily considered normal.

In the preparation of the curves 200 B chicks 21 days of age, 250 B chicks and 100 F chicks 15 days of age were used. (This study differs from that previously reported by Thayer, *et al.*[6]) By a series of different quantities (for Curve I, 0.4, 0.6, 0.8, 1.0, and 1.2 mg) we obtained responses which expressed in terms of percentage were plotted against the dosage (Fig. 1). These points fall upon a curve similar to that found by Trevan in his fundamental studies of bio-assay.

Chicks 15 days of age have been used in our later work for the following reasons: 1st, mortality is considerably less in attaining the age at which they are used (25% for the chicks 15 days of age and 50% for chicks 21 days of age); 2nd, food consumption is not as great; 3rd, the amount of care required by the chicks is reduced by the shorter period; and 4th, with the same caging facilities more assays can be conducted.

Our unit is that quantity of vitamin which produces a clotting time of 10 minutes or less in 50% of a group of ten or more chicks which has been fed for the 14 days immediately following receipt from the hatchery on a diet practically devoid of Vitamin K. The chart is so constructed that having the response in percentage the corresponding value in chick units is immediately apparent from the curve.

The accuracy of the method was checked by the administration of "unknowns" prepared from the extract which had been used in the preparation of the curves, groups of 10 chicks being used for each assay. In the assay of an unknown, it may be necessary to use several groups of chicks as it is necessary to find the dosage, the administration of which produces a response that falls on the curve,

TABLE I.
Accuracy of the Method.

Standard preparation administered as unknowns		Response	Found from standard curve		Error
mg	Chick units	% positive	mg	Chick units	%
0.65	0.8	50	0.80	1.0	+23
1.00	1.3	60	0.90	1.1	—10
0.95	1.2	50	0.80	1.0	—16
1.15	1.4	80	1.06	1.3	— 8
0.70	0.9	33*	0.58	0.7	—17

*1 chick died.

[6] Thayer, S. A., MacCorquodale, D. W., McKee, R. W., and Doisy, E. A., *J. Biol. Chem.*, 1938, **123**, CXX.

preferably near its midpoint. Examination of Table I shows that our method gives fairly good assays. However, a statistical study indicates that more accurate results can be obtained by using 20 or 30 chicks for each assay.

Our more recent experiences indicate that the degree of deficiency produced in 15 days in different shipments of chicks may show considerable variation. For this reason we determine the response of each batch of chicks to a standard preparation of Vitamin K. Over a period of the last 7 weeks the same dosage of the standard preparation was administered to 8 groups of approximately 10 chicks; the responses obtained were 40, 44, 50, 38, 50, 60, 70, and 50%. Moreover, we advise that before starting assays with a particular lot of chicks, that the clotting time of 10 or more should be determined to ascertain the degree of deficiency.

In order that other investigators may obtain an idea of the magnitude of our unit we find that one gram of the artificially dried alfalfa used in our work yields approximately 10 units of the vitamin. For these assays the alfalfa meal was exhaustively extracted with petroleum ether, the solvent distilled and the residue taken up in sesame oil.

In our early work in which the deficiency was less pronounced, the potency of our most highly purified concentrate was about 1000 units per milligram. In later work in which a more severe deficiency has been produced we find about 1000 units per milligram even though we are confident that the purity of the vitamin is definitely increased.

Summary. A curative method of assay of Vitamin K based on Trevan's principles of bioassay has been found to give satisfactory results.

10464

Inactivation of Vitamin K by Light.*

D. W. MacCorquodale, S. B. Binkley, R. W. McKee, Sidney A. Thayer and Edward A. Doisy.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Mo.

In an earlier note,[1] we reported the isolation of crystalline material from alfalfa which after 4 recrystallizations still had the activity ascribed to Vitamin K. Since our observations were still incomplete it was impossible to state with certainty that we had isolated Vitamin K. Some time after this work, it was found that although we could obtain crystals with approximately the same melting point, solutions of these crystals did not restore the coagulation time to nomal values. Frankly, we admit that we have been unable to duplicate the work reported last summer and, moreover, thus far do not have any explanation which appears to be satisfactory.

In seeking an explanation of our unexpected results, we recalled that Almquist[2, 3] had reported briefly on the loss of activity of preparations exposed to sunlight and on the stability of the vitamin exposed to artificial illumination. The effect of sunlight has been confirmed but in addition we have found that highly purified preparations dissolved in various solvents rapidly lose activity on exposure to the illumination from ordinary daylight bulbs. On the other hand, our crude extracts of alfalfa have been found to be quite stable, no special precautions regarding light being necessary. However, as the potency is increased to 500 or 1000 units (Thayer, *et al.*[4]) per milligram, the lability is such that decomposition has frequently occurred in spite of our precautions.

Experimental. Two preparations were used in our experiments on the effect of light: (a) a non-crystalline product obtained from alfalfa having a potency of approximately 1000 units per milligram and (b) a crystalline product (M.P. 50.5-52.0°) obtained from putrefied fish meal. The latter had been recrystallized 10 times from

* We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

1 Thayer, S. A., MacCorquodale, D. W., Binkley, S. B., and Doisy, E. A., *Science*, 1938, **88**, 243.

2 Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

3 Almquist, H. J., *J. Biol. Chem.*, 1937, **117**, 517.

4 Thayer, Sidney A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, Edward A., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 478.

various solvents without detectable alteration of its potency, which was approximately 600 units per milligram.

With both preparations dissolved in either ethyl alcohol or benzene, exposure to direct sunlight for 5 hours caused a destruction of more than 75% of the vitamin.

Exposure for 20 hours to light from a 300 watt daylight bulb at a distance of 4 feet produced loss of activity of solutions of the alfalfa extract dissolved in acetone or benzene. Solutions of the material in benzene, alcohol, and acetone were exposed to white light for 96 hours. In all cases destruction was extensive (Table I). In another experiment the fish meal preparation was dissolved in benzene and the solution was divided into 2 portions, one being kept in the dark and the other exposed during 3 working days (20 hours) to the artificial illumination used in the laboratory. The preparation kept in the dark was 10 times as active as the one exposed to light.

TABLE I.
Effect of Exposure to White Light, Prep. No. T3a (Prepared from Alfalfa).

	Solution kept in the dark				Solution exposed to the light				
			Response No. of chicks				Response No. of chicks		
Solvent	Chicks (15 days old) No.	Micrograms of material administered	Pos. +	Neg. —	Chicks (15 days old) No.	Micrograms of material administered	Pos. +	Neg. —	% loss of Vitamin K Potency
Benzene	6	1.0	3	3	10	6.0	1	9	>90
Ethyl Alcohol	5	1.0	2	3	5	2.0	1	4	>65
Acetone	5	1.0	3	2	10	4.5	1	8	>85

Control Chicks—(10)—clotting time >30 minutes.

Since in these experiments we were attempting to ascertain the cause of inactivation in our routine work of purification, oxygen was not excluded from the flasks containing the vitamin preparations. It is possible that oxygen may play a part in the destruction, but the same solutions kept in the dark did not show loss of activity.

10465

Time Factor in Retention of Nitrogen After Intravenous Injection of a Mixture of Amino-Acids.*

ROBERT ELMAN. (Introduced by Evarts A. Graham.)
(With the technical assistance of Ray Charnas.)

From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis, Mo.

As reported previously,[1] an acid hydrolysate of casein containing no tryptophane did not produce nitrogen balance when given intravenously. If tryptophane (and methionine) were added, nitrogen balance was achieved at once. In the course of these studies it was noted that if the tryptophane were injected several hours after the basic incomplete mixture its favorable effect on the retention of nitrogen is lost and that a negative nitrogen balance occurs, almost as if no tryptophane had been given. This observation was repeated and confirmed in several experiments which are described herein. Van Slyke, Cullen and McLean[2] found that the first amino-acids reaching the liver are deaminized and the nitrogen excreted very rapidly and that they do not await further absorption of additional amino-acids without which synthesis is impossible. They noted that this phenomenon, wasteful as it seems, occurred even when there was a great need of nitrogen because of starvation. A perhaps analogous time factor in the retention of nitrogen by carbohydrate was recently observed by Larson and Chaikoff.[3] In normal dogs they found that carbohydrates induced a retention of nitrogen from protein feeding only when the sugar was given not more than 4 hours before or 4 hours after the protein was ingested. The greatest retention occurred when the 2 were given simultaneously.

Three pairs of experiments were carried out; the data herein reported are from one of them, typical of all. Two dogs were observed simultaneously and nitrogen studies started only after a preliminary period of starvation and after the daily urinary output had reached a uniform level. Previous to the collection period the animals were started on twice daily gavage feedings. These consisted of 12½% sucrose solution containing sodium, potassium, calcium, and magne-

* Aided by a grant from the Louis B. Beaumont Fund.

1 Elman, Robert, PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 610.

2 Van Slyke, D. D., Cullen, G. E., and McLean, F. C., as quoted in Peters and Van Slyke, *Quantitative Clinical Chemistry*, 1931, Vol. 1, p. 394.

3 Larson, P. S., and Chaikoff, I. L., *J. Nutrit.*, 1937, **13**, 287.

TABLE I.

Day	Urine			Daily Avg				Remarks
	Vol., cc	Total N, g	Creat. N, g	Creat. N g	N Intake g	N Output g	N Balance g	
1	600	1.40	.072					Dog 04
2	680	1.43	.068					Wt 6.8 kg
3	670	.87	.068	.070	0	1.23	—1.23	
4	1020	1.77	.069					"Delayed" Injection
5	1160	2.56	.081					
6	940	2.18	.064	.071	1.50	2.17	— .67	
7	640	.76	.064					Recovery
8	900	1.07	.089					
9	720	.93	.072	.075	0	.92	— .92	
10	820	1.38	.081					Complete mixture
11	960	1.55	.071					injected
12	820	1.30	.064	.072	1.50	1.42	+ .08	
13	910	.72	.057					Recovery
14	700	1.09	.082					Wt 6.2 kg
15	750	.99	.074	.071	0	.93	— .93	
1	660	1.23	.058					Dog B70
2	780	1.34	.064					Wt 7.6 kg
3	660	.76	.056	.059	0	1.11	—1.11	
4	1220	1.18	.072					Complete mixture
5	1180	1.51	.082					injected
6	1280	1.07	.070	.075	1.50	1.25	+ .25	
7	880	.99	.072					Recovery
8	940	1.47	.084					
9	980	.68	.067	.075	0	1.05	—1.05	
10	1320	1.53	.086					"Delayed" injection
11	1180	1.57	.073					
12	1000	1.73	.069	.075	1.50	1.61	— .11	
13	840	.97	.070					Recovery
14	780	.78	.065					Wt 7.4 kg
15	730	.90	.075	.070	0	.88	— .88	

sium chloride (*i. e.,* Ringer's solution) and also an adequate amount of Vitamin B concentrate (Labco). This mixture yielded about 50 calories per kilogram and contained but an insignificant amount of nitrogen. Observations were made in 3-day periods as indicated in Table I. The amino-acids† were always given as a 10% solution with 10% glucose; about 10 to 15 cc were given each hour until the entire daily dose was injected. When the tryptophane (and methionine) were "delayed" they were injected 6 hours after the last injection of the basic mixture. The dose of tryptophane and methionine was 2% each by weight of the total amount of amino-acids previously injected. The period of observation was 15 days; 3 days each for preliminary data, for the "complete" injection, for recovery, for the "delayed" injection, and for the second recovery. The sequence was reversed in the second dog so that when the first one was receiving the "complete" mixture, the other was receiving the "delayed" injection. Kjeldahl determinations were used for the determination of nitrogen and the alkali picric acid method for creatinine.

From the data reported herein it is seen that positive nitrogen balance occurs only when the "complete" mixture is given at once. If tryptophane is injected 6 hours after the rest, this did not occur. It would seem from the present findings that the absence of one essential amino-acid, *i. e.,* tryptophane, at the time all the others are present in the blood and tissue prevents retention of nitrogen and presumably delays or even prevents synthesis of amino-acids to protein. These observations would seem to indicate that for the most efficient utilization of amino-acids they should be presented to the tissues at the same time, thus introducing a time factor in the biological value of amino-acids dependent normally on the rate of digestion and absorption from the intestinal tract. This time factor may play a rôle in regeneration of protein in any condition wherein absorption from the intestinal tract is delayed for one reason or another. It has been noted by Groen,[4] for example, that in the human with various diseases, notably Vitamin B deficiency, absorption of glucose is impaired. Cori[5] has discussed the fact that the general condition of an animal has a pronounced influence on the rate of intestinal absorption and that when 2 substances are fed simultaneously, the rate of absorption of each was reduced. This may be of great significance in view of the findings of Chase and Lewis[6] in white rats that there are

† The acid hydrolysate of casein was generously supplied by Mead Johnson & Co. Tryptophane and methionine were obtained from Eastman Kodak Co.

4 Groen, J., *N. E. J. Med.,* 1938, **218**, 247.

5 Cori, C. F., *Physiol. Rev.,* 1931, **11**, 144.

6 Chase, B. W., and Lewis, H. B., *J. Biochem.,* 1934, **106**, 315.

great differences in the rate of absorption of amino-acids from the intestinal tract; leucines, essential amino-acids, for example, were one-third as absorbable as glycine, a non-essential.

Summary. The injection of tryptophane (and methionine) 6 hours after the injection of an incomplete mixture of amino-acids lacking only tryptophane failed to induce positive nitrogen balance, whereas the injection of tryptophane (and methionine) simultaneously succeeded in doing so. It is inferred that retention of nitrogen is facilitated when all of a complete mixture of amino-acids is presented to the tissues at the same time.

10466 P

Inhibitory Effects of Adrenalin on Autonomic Function.

CHESTER W. DARROW AND ERNST GELLHORN.

From the Departments of Physiology and Psychiatry, University of Illinois, and Institute for Juvenile Research, Chicago.

Hoskins and Rawley[1] and Chu and Hsu[2] showed that adrenalin inhibits reflex sympathetic responses in the vascular system. The present study was undertaken to investigate the applicability of these observations to other autonomic reflexes.

In cats under urethane and chloralosane anesthesia the sympathetic nerve to the pupil and nictitating membrane of one eye was separated from the vagus and severed in the neck. Blood pressure and nictitating membranes were recorded by kymograph, and the 2 pupils and a galvanometer indicating the galvanic (sweating) reactions of the foot pads were photographed. Brachial plexus, sciatic and splanchnic nerves were freed, cut, and arranged for stimulation of their central ends. The autonomic responses of the respective pupils and nictitating membranes with and without sympathetic supply, the galvanic (sweating) reactions and blood pressure changes are compared (1) during secretion of adrenine, (2) under the influence of adrenalin perfusion, and (3) after adrenalectomy.

The kymographic and photographic records show that during infusion of adrenalin (1:100,000 to 1:250,000, 1-3 cc/min) sympathetic reflex responses decrease. This is evident from the decreased reflex responses of the normally innervated pupil and nictitating

1 Hoskins, R. G., and Rowley, W. N., *Am. J. Cliniol.*, 1915, **37**, 471.

2 Chu, L. W., and Hsu, F. Y., *Quart. J. Exp. Physiol.*, 1938, **27**, 307.

membrane and decreased sweating of the foot pads. The blood pressure is generally slightly elevated and the pressor response to afferent stimuli is reduced. The reflex (inhibitory) dilatation of the sympathectomized pupil is increased. The "damping" effects were generally reduced following ligation of both adrenals. They could be overcome by small injections of metrazol which, according to our previous investigations,[3] greatly accentuates sympathetic responses. In like manner hyperreflexia induced by metrazol could be reduced by injecting adrenalin. The action of adrenin liberated under various conditions in the narcotized animal is similar to that of adrenalin perfusion.

10467 P

Control of "Nose-Picking" Form of Cannibalism in Young Closely Confined Quail Fed Raw Meat.*

CHARLES C. BASS.

From the Laboratory of Clinical Medicine, School of Medicine, Tulane University of Louisiana, New Orleans.

When young quail are kept crowded together, in considerable numbers, in very small brooder pens, they soon develop the "nose-picking" form of cannibalism. It may start at any age from about the second up to the sixth or seventh week. It seldom or never starts after the eighth week; or, if it has already started, it usually stops at about that time.

One or more birds (usually the larger and stronger ones) start pecking the upper bills of other birds (usually the smaller and weaker ones). The nostril is the point of injury. The wound is small and superficial at first but it is enlarged and deepened from the repeated picking by the offending bird or birds, until, within 2 or 3 days, it is quite serious. This weeping and bleeding wound invites further injury. The bill at this point may be cut half through. The victim is in pain, droops around, eats and drinks very little and becomes weak. The weaker he grows the more the other birds attack him. Usually he is soon trampled and smothered to death by the other stronger birds. Within a few days a large percent of all the birds in

3 Gellhorn, E., and Darrow, C. W., in press.

* This work has been aided by a grant from Mr. A. B. Freeman and Mr. Robert Newman, of New Orleans.

the pen may be injured. If nothing is done to stop it, most of them may die. Birds that recover usually have badly deformed bills and are worthless for breeders.

This trouble occurs only when the quail chicks are brooded in artificial brooders. It does not occur if they are kept in large pens, especially if the pens are on the ground where there is plenty of trash and litter for them to pick at and scratch in.

Many quail breeders believe this form of cannibalism is due to some fault or deficiency in the diet. Animal protein is thought to be the element needed and an attempt to supply the deficiency is made by including in the feed mixture varying proportions of dried meat meal, fish meal or milk products. However, no diet heretofore employed has been found adequate to entirely prevent the trouble whenever the birds are closely confined and crowded in the brooder pens.

For experimental purposes I have used wire bottom pens, 15″x21″, in which from 25 to 50 birds are kept through the period during which this form of cannibalism occurs. Under such very crowded conditions they always develop "nose-picking" and all but a few of the entire lot are finally killed. Several different commercial game bird or poultry feeds have been tried; and also feeds which I have made up according to different formulæ. None of them prevents the trouble. On the other hand, I have found that if, in addition to their other feed, the birds are given all the raw meat they will eat, and a plentiful supply is kept before them all the time, "nose-picking" does not occur; or, if it has already started, it soon stops. Ground beef was found effective for this purpose.

It is sometimes necessary to withhold all other food temporarily, to force the birds to eat enough raw meat to prevent this form of cannibalism; especially to control it after it has already started. Birds of this age, once they learn the taste of it, eat the raw meat ravenously and will consume even more than grown quail, although they are only about one-fifth as large.

10468 P

Plasma Specific Gravity and Control of Fluid Administration in Artificial Fever.*

HERBERT R. BROWN, JR., AND WILLIAM F. CLARK. (Introduced by W. S. McCann.)

From the Department of Medicine, School of Medicine and Dentistry, University of Rochester, and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

Studies on plasma-specific gravity by Moore and Van Slyke revealed that a linear relationship exists between the total proteins and the specific gravity of plasma.[1] These observations were originally carried out on normal adult males and females, on patients with nephritis in varying degrees of edema and dehydrated individuals.

Table I represents a new classification derived from many of their original ranges, and the results of over 1,000 determinations by us in similar types of patients.[1, 2] The application of this table is thus made available to the control of fluid administration. We have been able to show that the relative amounts of dehydration and hydration can be accurately estimated by determination of plasma specific gravity. The need for estimation of adequate fluid balance in artificial fever therapy has long been wanting. The results of our observations illustrate that by this method one can accurately control the body fluid needs.

In over 50 normal adult males and females the plasma levels remained in the range of 1.0255-1.0288. The highest reading in dehydration thus far obtained was 1.0328 shortly after which the patient died. The lowest reading obtained was 1.0169 in a case of terminal nephritis.

The blood, 5 cc in amount, is obtained from the patient's ante-

TABLE I.†

Classification	Plasma Specific Gravity	Total Proteins
Dehydration	>1.0288	>7.5
Normal	1.0255-1.0288	6.3-7.5
Low Normal and Overhydration	1.0235-1.0255	5.6-6.3
Threshold Levels (at which edema may appear)	1.0227-1.0235	5.3-5.6
Edema	<1.0227	<5.3

† A problem now under investigation reveals that an important seasonal variation exists, which will be dealt with in a later paper.

* Appreciation is extended to Drs. Stafford L. Warren and Nathaniel Jones for their advice and cooperation in this investigation.

1 Moore, N. S., and Van Slyke, D. D., *J. Clin. Invest.*, 1930, **8**, 337.

2 Peters, J. P., and Van Slyke, D. D., *Quant. Clin. Chem.*, **1**, pp. 662, 682.

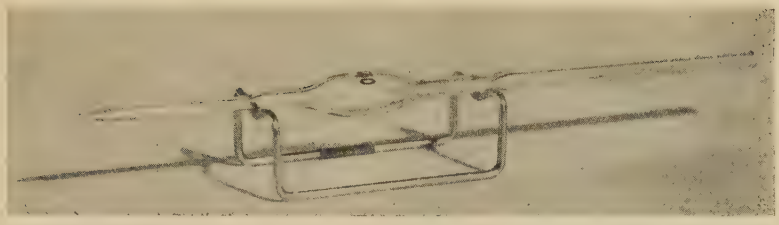


FIG. 1.
A 1 cc plasma pipette.

cubital vein without stasis to avoid increasing the plasma protein concentration.[3] This is delivered to a bottle containing a mixture of 4 mg of potassium oxalate and 6 mg of ammonium oxalate. This anticoagulant assures stability in the size of the red blood cells by eliminating fluid interchange and hemolysis. The blood is then centrifuged at 2200 revolutions per minute for 3 to 4 minutes.[4] Plasma rather than serum is used to include the weight of fibrinogen. These precautions thus reduce sources of error in blood collection.[5]

The method employed in this experiment is of the gravimetric type using special weighing pipettes.[6]

They much resemble blood pipettes and have volumes of 1 cc and 0.5 cc. Each has an outlet which measures .0002 cm^3 in diameter. The fluid once measured to the meniscus will remain there, since the narrow outlet becomes partly sealed by coagulation, and the fluid column immobilized by capillary adhesive forces as well. Once the level has been reached the pipettes may be inverted, tilted, and left for reasonably long periods of time without changes in weight or meniscus, since errors due to evaporation and mechanical loss are eliminated.

The plasma-filled pipette is then weighed and recorded to the fourth place. The temperature of the plasma is taken while the filled pipette is being weighed. The pipettes are cleaned and dried by suction with water and acetone, but not ether.

Calculations.

Formula:

$$\text{Weight of Plasma in Pipette} \pm \frac{\text{Capacity of Container in cc}}{2} \times \text{Temperature Factor} = \text{Weight of Distilled Water in Pipette at } 20°$$

Plasma Specific Gravity.

The temperature correction factor to be added for readings above 20°C and subtracted for readings below 20°C.

3 Rowe, A. H., *J. Lab. and Clin. Med.*, 1915-1916, **1**, 485.

4 Heller, V. G., and Paul, Henry, *J. Lab. and Clin. Med.*, 1934, **19**, 777.

5 Peters, J. P., Eisenman, A. J., and Bulger, H. H., *J. Clin. Invest.*, 1925, **1**, 435.

6 Pregl, *Wiener Klinische Wochenschrift*, 1925, **38**, 663.

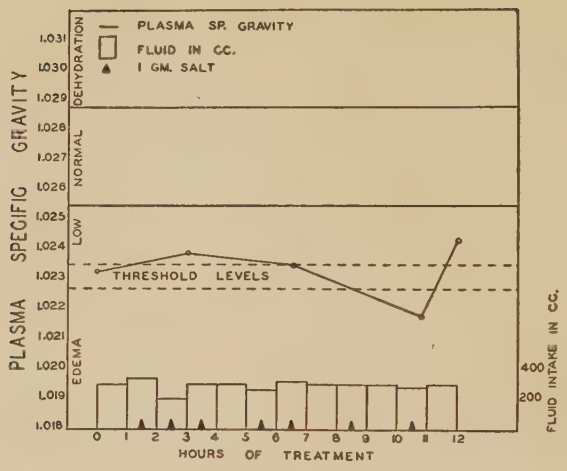


FIG. 2.

This patient was a 37-year-old woman with general paresis treated for 12 hours at 41.5°C. The plasma levels remained in the overhydration range and for several hours during treatment gross edema was present. A total of 3550 cc of water and 7 g of salt were used.

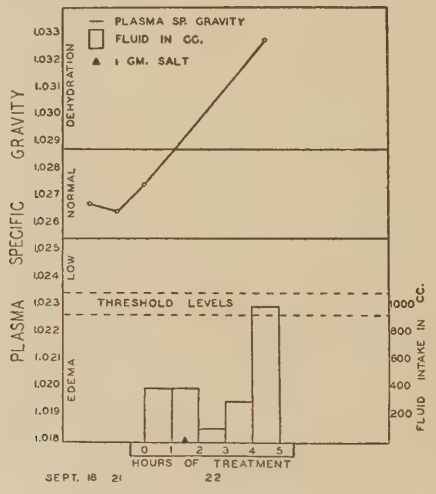


FIG. 3.

This patient was a 45-year-old man treated for general paresis at 41.5°C. Since his fluids were poorly stored, the dehydration factor constituted the major problem here. Just after the temperature reached 41.5°C, the patient's condition became critical with ensuing collapse, and coma, after which he expired. A total of 2100 cc of water and 1 g of salt was used.

The weight of the dry, clean pipette is subtracted, in the above formula, from the plasma-filled pipette. Since the volume is stationary, only plasma need be weighed, except for necessary periodic restand-

FIG. 4.

This case represents a 23-year-old man, treated for 48 hours at 39.5°C for infectious arthritis. Fluid and salt administrations were increased and decreased at various intervals as indicated. Dehydration never became a factor in this controlled case. Early signs of overhydration and edema between the twentieth and twenty-fourth hour were signals for reduction of fluids and salts. From this period on normal ranges were entered and maintained with a maximum of comfort for the patient. General condition excellent at the end of treatment.

ardizations with distilled water to check changes following cleaning processes.

The capacity of the pipette can be obtained directly from the weight of distilled water as recorded in Landolt and Börnstein's Tabellen.[7] The temperature factor is obtained from the graph in Peters and Van Slyke,[7] which corrects to 20°C in terms of which all values are expressed. The total protein content of plasma may then be computed from the specific gravity by the formula:

$$\text{Total Protein/100 cc plasma} = (343 \times (\text{Sp.gr.} - 1.007)).$$

This method has been selected because of its simplicity and greater

[7] Peters, J. P., and Van Slyke, D. D., *Quant. Clin. Chem.*, **2** Methods, pp. 5, 690, 691.

accuracy. As compared with Kagan's[8] modification of Barbour and Hamilton's[9] falling drop method for the determination of plasma specific gravity, we have found that an entire series of determinations can be done with greater accuracy and rapidity. This method eliminates the use of nomograms, except for temperature correction, and the chance of error in timing of drops and controlling of standards.

This method of estimating body fluid status has been applied successfully to patients during artificial fever therapy. Figs. 2 and 3 reveal situations which were encountered before specific gravity was routinely measured and the fluid administration regulated by its interpretation. The data from Figs. 2 and 3 represent the extremes of a series of cases during the fall of 1938. No analyses or interpretations of the first few cases were made until a sufficient number had been obtained. Thus fluid regulation was not controlled in these early cases by this gravimetric method. Fig. 4 is that of a prolonged 48-hour treatment and indicates how well adequate hydration can be controlled by repeated determinations. In this case an endeavor was made to keep the specific gravity as nearly as possible at 1.0255.

Conclusions. 1. The use of special gravimetric pipettes introduces a new, rapid and accurate method for determining plasma specific gravity. 2. The measurement of specific gravity in artificial fever assures proper regulation of body fluid balance and eliminates the dangers of dehydration and over-hydration during the course of treatment. Many of the hazards of this form of therapy are thus reduced when proper levels are maintained or, in difficult situations, the treatment is stopped.

---

[8] Kagan, B. M., *J. Clin. Invest.*, 1938, **17**, 369.

[9] Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

10469 P

Clumping of Extracellular Encapsulated Pneumococci in Sputum for Control of Serum Therapy.

ARTHUR W. FRISCH.* (Introduced by W. O. Nelson.)

From the Department of Bacteriology, Wayne University College of Medicine, Detroit, Michigan.

In a previous report[1] it was shown that clumping of encapsulated extracellular diplococci in pneumonic sputum could either occur spontaneously or be induced by the intravenous administration of specific horse- or rabbit-serum. Further evidence that antibody is responsible for the clumping has been obtained from a patient infected with type 8 pneumococci. Nine hours after the intravenous injection of 160,000 units of rabbit serum the clumped pneumococci in the sputum showed "*Quellung*" in preparations to which methylene blue alone had been added. At the present time, clumping has been induced after the administration of Types 1, 2, 3, 5, 7, and 8 serum. Spontaneous clumping, now believed to represent evidence of developing active immunity, has been observed in patients infected with Types 1, 2, 3, 4, 5, 7, 8, 12, 18, 19, 24 and 25 pneumococci.

The induction and maintenance of clumping has a direct bearing upon the outcome of the pneumonia, as shown in Table I. Sixty-nine roentgenographically proved cases of pneumonia were followed from the sputum by the method previously described.[2] All of the

TABLE I.

			No clumping induced		Induced but not maintained		Induced and maintained	
Type	Cases	Bacteremia	Rec.	Died	Rec.	Died	Rec.	Died
1	20	7	0	1	0	0	18	1
2	26	8	0	2	0	3	17	4
3	5	2	0	2	0	0	3	0
5	2	1	0	0	0	0	2	0
7	11	5	0	1	0	0	9	1
8	5	2	0	1	0	0	4*	0
	69	25	0	7	0	3	53	6

* Patient subsequently developed a type 2 pneumonia during convalescence and expired.

* Aided by a grant from the Commonwealth Fund to the Michigan Department of Health Laboratory. Acknowledgments are due Doctors A. E. Price, G. B. Myers, H. L. Clark, and O. A. Brines for their criticisms and permission to use clinical and laboratory data.

1 Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 473.

2 Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 48.

patients received either horse or rabbit antipneumococcic serum; 10 were given sulfanilamide† in addition to serum.

The 10 patients who either failed to develop or to maintain clumping, expired. In 47 of the 53 serum-treated patients who recovered, clumping in the sputum was induced and maintained throughout the acute stages of the disease. The remaining 6 recovered cases showed clumping of extracellular pneumococci in specimens of sputum obtained before serum therapy was instituted. Although clumping was induced and maintained in 6 patients, they failed to recover. One (Type 2) in the author's opinion, died of asphyxia. Another (Type 2) was convalescing and expired shortly after an attempted aspiration of an interlobar empyema. A third (Type 7) was suspected of having bacterial endocarditis and developed pneumonia and meningitis in the hospital. The fourth patient (Type 2) showed clumping a few hours before death. In the remaining cases, one (Type 2) was complicated by secondary infection from a decubitus ulcer and the other (Type 1) by delirium tremens.

In 26 of the above patients the dosage of serum was controlled by using the appearance of clumps of encapsulated pneumococci in the sputum as an index of adequate therapy. Three (Types 2, 3, 8) received large amounts of serum but clumping either failed to develop or was not maintained, and they expired. In the remaining 23 cases, clumping was successfully maintained and the patients recovered. In 3 of them (Types 1, 5, 7) no dramatic drop in temperature and pulse occurred, although clumping had been induced and was being maintained. All 3 patients recovered without receiving additional serum.

The sputa of 4 of the 23 patients contained only a few encapsulated extracellular pneumococci which were already clumped before treatment was begun. In these cases it was felt that regardless of the amount of serum given, the patients would recover. They received the following therapy. The first case (Type 1) was treated with 100,000 units of horse- and the second (Type 1) with 30,000 units of rabbit-serum. The third patient (Type 7) received 20,000 units and the fourth (Type 7) 10,000 units of antipneumococcic horse serum. A fifth case with a similar sputum-picture (Type 8) was given no serum. Under routine therapy each of these patients would have received from 100,000 to 250,000 units, yet with no serum at all or with doses ordinarily considered inadequate, all 5 of the patients responded by crisis. These preliminary data are encouraging enough to warrant the further study of clumping of encapsulated pneumococci in the sputum as a method for the control of serum therapy.

† The effect of sulfanilamide is largely bacteriostatic and does not influence the clumping. Therapy with this drug will be discussed in a future communication.

10470 P

Occurrence of Urinary Calculi in Inbred Strain (C3H) of Mice Treated with Estrogen.

EDWARD L. BURNS AND JOHN R. SCHENKEN. (Introduced by Kenneth L. Burdon.)

From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.

We have observed in male mice of the C3H* strain many of the pathologic changes in the genitourinary tract reported by previous investigators[1, 2, 3] to follow the injection of large doses of estrogen. We have also observed the occurrence of urinary calculi.

One hundred fifty-one male animals were injected subcutaneously at the age of 2 weeks with Progynon-B (estradiol benzoate in a solution of sesame oil). Twenty-three animals were given 2 doses each of 1500 rat units on alternate days. One hundred fourteen were injected with 100 rat units at weekly intervals for 4, 8, 12, 16, and 20 weeks. In both groups treatment was terminated at the end of these intervals. In 14 other mice similar weekly injections were continued for periods varying from 21 to 35 weeks. Sixty-eight female and 28 male C3H mice were used for controls. The diet consisted of fresh Purina dog chow, with lettuce approximately once a week. Water was always available through a drinking tube.

In 41 experimental animals which died or were sacrificed the presence of calculi was determined by dissection of the urinary tract and roentgen-ray study of the dissected specimens. All the calculi proved radio-opaque. Since an accurate antemortem diagnosis of stones had frequently been made by palpation of the bladder through the abdominal wall, a census was taken of the entire colony, and roentgenograms were made of all mice in which stones could be palpated.

Calculi occurred in 4 (4.1%) of the 96 control mice, and in 50 (33.1%) of the 151 injected mice. All animals under 5 months of age were discarded in our additional calculations for 3 reasons: (1) Only 5 of the 55 injected mice less than 5 months old developed calculi. (2) The incidence of calculi in the injected animals rose from 14.3% in the fifth to 66.6% in the sixth month. (3) None of

* The parent animals were obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine.

1 Lacassagne, A., *Compt. rend. Soc. de biol.*, 1933, **113**, 590.

2 Burrows, H., and Kennaway, N. M., *Am. J. Cancer*, 1934, **20**, 48.

3 Rusch, H., *Endocrinology*, 1937, **21**, 511.

the control animals under 5 months of age developed calculi. Of the 20 male and 43 female controls in this selected age group, 4 (6.3%), all females, developed stones. Of the 96 injected males in this age group, however, 45 (46.8%) developed calculi.

The incidence of stones varied according to the dose of estrogen administered (Table I). The mice injected with 100 rat units for more than 20 weeks, and those given a total of 3,000 rat units in 2 injections on alternate days, showed a relatively low and almost equal incidence of stones. Mice which had had 9 to 20 weekly injections of 100 rat units showed the highest incidence.

TABLE I.

No. weekly doses 100 rat units estrogen	4	5-8	9-12	13-16	17-20	Above 20	3000 in 2 doses
No. animals with stones	3	7	7	9	11	3	5
% animals with stones	23	58	78	81	73	23	22

The data now available would seem to indicate that stones appear at an earlier age in injected than in control animals. The average age of control animals above 5 months of age without stones was 11 months, 7 days, and of the 4 female controls with stones 11 months, 17 days. The average age of the injected male mice without stones, however, was 8 months, 25 days, and of the 45 with stones 8 months.

The location and appearance of the calculi differed in the injected and the control mice. In the latter urinary stones were found only in the bladder. In the injected mice most of the stones were in the bladder, but occasionally they appeared in the urethra, ureters, and kidneys.

In the injected mice all the calculi were white, chalky in appearance, moderately soft, and sometimes laminated. Usually from 3 to 10 were present in the bladder. The individual stones varied in size from tiny, gravel-like fragments to concretions 0.5 cm in diameter. Often several calculi were cemented together with a soft, chalky material or a tenacious mucoid substance, to form a cast of the entire bladder. In control animals the stones were hard, smooth, discrete, and uniformly 2 to 3 mm in diameter, and not more than 4 were present in any instance.

Summary. Urinary calculi occurred in 33.1% of the 151 strain C3H male mice treated with estogren, and in 4.1% of 96 controls. The highest incidence was noted in mice 5 months of age or older, which had received from 9 to 20 weekly injections of 100 rat units. The calculi appeared at an earlier age in the treated animals.

10471

Effect of Brief Experimental Hyperthyroidism on Reproduction in the Rat.

C. P. Kraatz. (Introduced by L. B. Nice.)

From the Department of Physiology, Chicago Medical School.

The experiment here reported endeavors to determine whether a short, vigorous treatment with thyroid substance will stimulate ovarian function in the rat, without the marked disturbance of reproduction observed after lengthy treatment.[1, 2, 3]

Thyroid substance (Wilson) mixed with a few drops of water was fed by medicine dropper to 35 virgin female rats (Sprague-Dawley strain) 4 to 6 months old. Daily dosages of 0.25 and 0.3 g per rat were given for periods of 3, 4, and 5 days. Animals were allowed to mate with normal males at the first estrous period after the last feeding. Thirty-five untreated littermates served as controls. The data on reproduction in the latter throughout the year approximate the figures for the local and parent colonies.[4]

The experimental animals fall into 2 groups, Series A, treated in June, and Series B, treated in April, October, and December. Results are summarized in Table I.

Series A typifies the more pronounced effect of thyroid administration observed during hot weather: the loss of body weight is greater

TABLE I.
Breeding Experiments with Thyroid-fed Female Rats and Littermate Controls.

	Series A	Series A Controls	Series B	Series B Controls
Animals exposed to males	9	6	26	29
Total litters resulting	8	6	26	24
Avg preliminary wt of mothers, g	218.1	230.2	219.6	215.5
" loss of wt. on thyroid, %	8.1	—	5.8	--
" No. of young per litter	8.25	10.0	12.3	9.0
" wt of young at birth, g	5.78	5.6	5.36	5.92
" age at eruption of upper incisors, days	7.6	8.0	8.5	8.1
% of young living 21 days*	51.1	91.6	71.8	69.4
Avg wt of young at 21 days of age, g (determined only on litters of 6)	36.8	42.2	37.5	37.8

Series A—fed thyroid during June, 1938.
Series B—fed thyroid during April, October and December, 1938.
*Litters reduced to 6 at an early age.

1 Gudernatsch, J. F., *Am. J. Physiol.*, 1915, **36**, 370.

2 Hayashi, H. H., *Bull. de l'Acad. de Méd.*, 1929, **101**, 115.

3 Weichert, C. K., *Physiol. Zool.*, 1930, **3**, 461.

4 Personal communication from Evan C. Holtzman of Sprague-Dawley, Inc.

than that of Series B on the same dosage. The somewhat smaller litters and markedly reduced survival of the young in Series A suggest the reproductive disturbances of severely hyperthyroid animals.

Series B, on the other hand, shows a stimulation from thyroid treatment during cooler weather. The litter size is definitely higher than in the controls, accompanied by a slightly lower weight of the young at birth. Survival in Series B, though low, is the same as in the controls and development proceeds at an essentially normal rate as evidenced by the figures on tooth eruption and 21-day weight. Litters were reduced to 6 within 3 or 4 days after birth so comparison of the figures is justifiable.

The frequency distribution of all litters according to size is shown in Fig. 1.

Six of the 26 litters in Series B are larger than the maximum control litters with only 3 falling below the control average of 9.2, while only 3 of the 30 control litters are larger than the 12.3 average of Series B.

As mentioned, an attempt was made to induce conception as early as possible after thyroid treatment. Table II lists the litters of Series B according to the interval between the end of thyroid administration and fertilization.

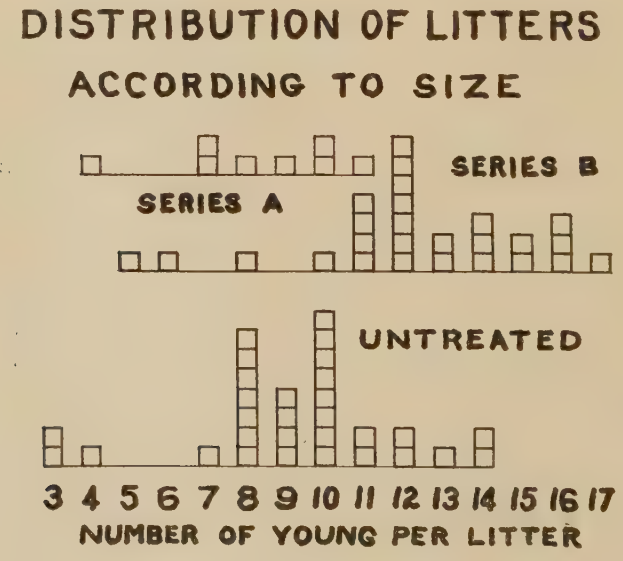


FIG. 1.

Frequency distribution of all litters. Series A received thyroid treatment in June, Series B in April, October and December. Untreated animals from both series are combined.

TABLE II.
Distribution of Series B Litters Relative to Thyroid-feeding Period.

	Day sperm was found in the vaginal smear; "0" is the last day of feeding									
	0	1	2	3	4	5	6	—	—	22
No. of young in litter	6	11	11	8	12		5			12
		12	11	10	14		11			
		12	14	12						
		13	16	12						
		14	16	12						
		15	17	13						
		15		16						
Avg litter size		13.1	14.2	11.9	13.0		8.0			

The first 4 days following thyroid feeding appear to be an "effective period," for the 22 rats bred during days 1 to 4 delivered an average of 13.0 young per mother. The present experiment, however, does not necessarily determine the extent of this period.

The range of dosages used varied little in its effects, except possibly with the highest dose. Of the 22 rats fertilized during the effective period, 2 receiving 0.3 g of thyroid daily for 3 days averaged 12.0 per litter; 7 receiving 0.3 g for 4 days averaged 12.4; 8 receiving 0.25 g for 5 days averaged 12.6, and 5 receiving 0.3 for 5 days averaged 14.8.

Of another series of 8 animals receiving 0.4 g of thyroid daily for 10 days, only 3 delivered litters (15, 8, and 5 young) and 2 of these failed to nurse them.

Kunde, Carlson and Proud[5] record an observation suggestive of the results reported here. In rabbits under continued thyroid treatment begun before mating, they found at laparotomy early in pregnancy an abnormally high number of fetuses, although few were born alive.

An augmented liberation of ova may or may not be a factor in the increased litter size following limited thyroid treatment. That the number of ova is the determining factor in litter size seems improbable in the light of the report of Long and Evans[6] that they found an average of 9.6 ova in the tubes of their rats, while the average litter size of the colony was 6.9. The strain of rats used in the present experiment, averaging 9 per litter, may conceivably liberate an average of 13 ova.

A tentative and partial explanation better supported by the available evidence is that the hyperthyroidism stimulates the release of luteinizing hormone from the anterior pituitary. The consequent

[5] Kunde, M. M., Carlson, A. J., and Proud, T., *Am. J. Physiol.*, 1929, **88**, 747.
[6] Long, J. A., and Evans, H. M., *Memoirs of the Univ. of Cal.*, 1922, v. 6.

increase in secretion by the ovary provides for the maintenance in the uterus of a higher number of embryos. Van Horn[7] found that the gonad-stimulating power of the hypophysis was increased in female rats hyperthyroid for several weeks. Halpern and Hendryson,[8] after brief daily treatment of non-pregnant adult rats with 0.5 g of thyroid, observed enlarged ovaries with increased corpus luteum development and suggested an activation of the "lutein-stimulating" hormone of the anterior pituitary. Hayashi[2] made similar observations with a lower dosage and described degeneration of the corpora lutea under long treatment.

Summary. The daily administration of 0.25 to 0.3 g of thyroid substance to adult female rats for 3 to 5 days during April, October and December, followed by mating within 4 days to normal males, resulted in an average litter size of 13.0, as compared to 9.0 in untreated littermates. Similar treatment during hot weather or an increase in thyroid amount proved deleterious to reproduction.

10472 P

Significance of Sex Hormones in Tanning of the Skin of Women.

JAMES B. HAMILTON.* (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine.

As illustrated in castrated and eunuchoid men, tanning of the human skin is abetted by the presence and negated by the absence of effective levels of male hormone substances in the body tissues and fluids.[1] Pending completion of analyses of urinary hormone titers and spectrophotometric study of skin pigmentation, the present report will serve to indicate that both the tanning process and its dependence on hormones for photograph-like "development" are (a) somewhat similar in women to those described for men, (b) capable of induction in women by male hormone substance, and (c) influenced by female as well as by male hormones.

---

7 Van Horn, W. M., *Endocr.*, 1933, **17**, 152.

8 Halpern, S. R., and Hendryson, I. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 263.

* This investigation was supported in part by a grant from the International Cancer Research Foundation.

1 Hamilton, James B., and Hubert, Gilbert, *Science*, 1938, **88**, 481.

Observations were made of the skin color of 5 women who received estrogenic or androgenic hormone substance. All complained of attacks of hot flushes and, with variation from individual to individual, some degree of additional malaise. Two of the women were considered to have reached the menopause and had not menstruated for the past 6 months. Two others had undergone bilateral ovariectomy, and the fifth had a hysterectomy done late in the third decade.

Three of the women received intramuscular injections of estrone† from 2 to 3 times weekly, a total of 14,000, 20,000, and 25,000 I. U., respectively, being administered over a period of one month in doses varied according to the degree of control over the hot flushes. The other 2 women were given testosterone propionate† by Doctor Edward Cravener as a possible treatment of arthritic conditions which had failed to respond to the customary therapeutic measures. The dose was 20 mg of testosterone propionate in 1 cc of peanut oil given intramuscularly from 1 to 3 times weekly for 3 weeks.

Increased coloration of the skin appeared within a few days in the women receiving either male or female hormone substances. All 5 women exhibited increased pigmentation of the skin of the body. One of them showed a distinct white tracing where the shoulder straps of a sun-tan suit had been worn. Since the woman had not worn a sun-tan suit for at least 2 months, it is indicated that the exposure to sunlight that was partly responsible for the tan of the body may have occurred before and not after the administration of the hormone. The hands, head and neck of the patients assumed a deeper tan than the body, possibly due in part to a greater exposure of these areas before and after administration of the hormone. Marked increase in pigmentation occurred on areolae and vulvae.

Vasodilation of the skin was especially striking in both of the women receiving testosterone propionate. One also commented upon the assumption of a darker color of the hair and upon a "weather-beaten" facial appearance, the latter seemingly due in part at least to increased prominence of the facial muscles.

† Estrone under the trade name Theelin was furnished through the courtesy of Parke, Davis and Co.; testosterone propionate under the trade name Perandren, through the courtesy of the Ciba Pharmaceutical Company, Inc.